MOVEMENT AND FATE OF SOLUTES IN A PLUME OF SEWAGE-CONTAMINATED GROUND WATER, CAPE COD, MASSACHUSETTS: U.S. GEOLOGICAL SURVEY TOXIC WASTE GROUND-WATER CONTAMINATION PROGRAM

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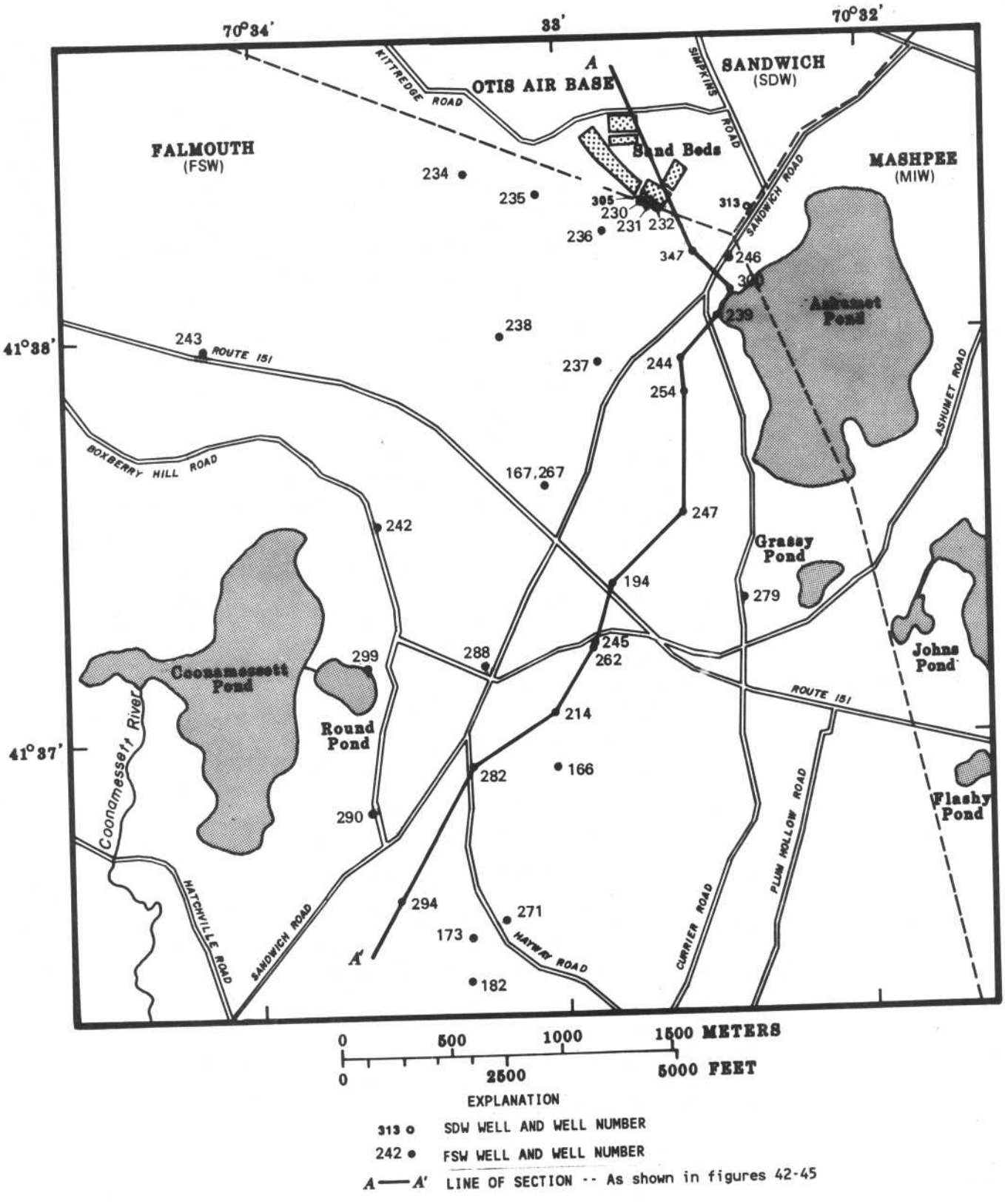

Figure 41.--Location of observation wells.

Table 15.--Water sample analyses, Cape Cod, Massachusetts
July-August, 1983

[A dash indicates no analysis was available]

Well number	pH	Specific conductivity (umhos)	Dissolved oxygen[3] (mg/L)	Temperature (°C)
FSW				
166-67	5.8	100	0.8	9.5
167-55	6.0	48	10.8	9.0
173-69	5.9	122	--	10.0
182-69	5.7	80	--	9.0
194-57	5.6	145	4.4	11.5
214-60	5.6	83	--	13.5
230-48	5.4	115	--	10.5
231-57	--	145	--	12.0
232-58	5.9	153	--	12.0
234-99	6.4	122	--	12.5
235-94	--	81	--	11.0
236-106	4.9	126	--	10.0
237-88	6.4	128	--	14.0
238-106	5.2	95	--	10.0
239-64	6.6	190	.0	13.0
242-77	5.4	51	--	10.0
243-45	6.2	38	--	9.0
244-90	6.6	230	--	11.0
245-25	4.7	200	--	10.0
246-35	5.6	152	--	12.0
247-70	5.4	120	2.6	--

[3]The dissolved oxygen data were collected in 1979 (LeBlanc, 1982).

Table 15.--Water sample analyses, Cape Cod, Massachusetts
July-August, 1983--Continued

[A dash indicates no analysis was available]

Well number	pH	Specific conductivity (µmhos)	Dissolved oxygen[3] (mg/L)	Temperature (°C)
FSW				
254-216	6.9	59	.1	10.0
254-168	6.4	115	.0	10.0
254-140	6.3	175	--	10.5
254-107	6.2	235	.0	10.5
254-72	6.7	225	.0	11.0
254-54	6.3	220	.0	10.5
254-26	4.9	70	8.1	10.0
262-159	6.7	125	--	10.0
262-85	5.9	255	.0	11.0
262-69	5.7	200	.0	11.0
262-41	5.0	90	8.0	10.0
267-155	6.7	122	--	9.0
267-136	6.0	95	8.2	9.0
267-111	5.9	120	.0	9.0
267-88	5.5	195	.0	9.0
271-165	6.9	125	--	9.0
271-141	6.9	132	--	9.0
271-85	5.0	150	--	10.0
271-41	5.2	55	--	10.0
279-86	--	76	4.3	11.0
279-61	--	73	7.3	11.0

[3]The dissolved oxygen data were collected in 1979 (LeBlanc, 1982).

Table 15.--Water sample analyses, Cape Cod, Massachusetts
July-August 1983--Continued

[A dash indicates no analysis was available]

Well number	pH	Specific conductivity (µmhos)	Dissolved oxygen[3] (mg/L)	Temperature (°C)
FSW				
282-123	5.9	143	.8	9.0
282-94	5.7	208	.1	9.0
282-70	5.4	215	.2	10.0
282-49	6.0	100	11.4	9.0
288-97	6.3	142	.0	9.0
290-91	5.9	90	.3	10.0
294-89	6.0	139	.4	9.5
299-20	5.2	76	--	10.0
300-30	6.5	410	.0	11.5
300-10	5.2	138	5.9	13.0
347-67	6.3	302	.0	10.0
SDW				
305-32	6.2	122	--	9.5
313-60	--	100	10.0	10.0

[3]The dissolved oxygen data were collected in 1979 (LeBlanc, 1982).

Table 15.--Water sample analyses, Cape Cod, Massachusetts July-August, 1983--Continued.

[A dash indicates no analysis was available]

Well number	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	NH_4^+ (mg/L as N)	Dissolved organic carbon (mg/L)
FSW				
166-67	0.92	<0.06	<0.10	1.3
167-55	<.06	<.06	<.10	1.0
173-69	2.2	<.06	<.10	1.0
182-69	1.6	<.06	<.10	1.0
194-57	1.7	<.06	1.6	2.3
214-60	2.3	<.06	.11	1.4
230-48	3.4	<.06	<.10	2.0
231-57	6.4	<.06	<.10	3.0
232-58	4.6	<.06	<.10	3.2
234-99	2.2	<.06	<.10	.9
235-94	1.3	<.06	<.10	.9
236-106	1.1	<.06	<.10	1.1
237-88	2.6	<.06	3.8	1.1
238-106	2.0	<.06	<.10	<1.0
239-64	<.06	<.06	7.0	1.9
242-77	.21	<.06	<.10	.9
243-45	.29	<.06	<.10	.8
244-90	<.06	<.06	10.7	1.8
245-25	1.2	<.06	<.10	1.0
246-35	5.0	<.06	<.10	2.0
247-70	1.4	<.06	.60	1.0
254-216	<.06	<.06	<.10	1.0

Table 15.--Water sample analyses, Cape Cod, Massachusetts July-August, 1983--Continued.

[A dash indicates no analysis was available]

Well number	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	NH_4^+ (mg/L as N)	Dissolved organic carbon (mg/L)
FSW				
254-168	<0.06	<0.06	<0.10	1.1
254-140	.31	<.06	<.10	1.9
254-107	1.0	<.06	<.10	2.3
254-72	.08	<.06	9.1	2.8
254-54	.57	<.06	7.6	3.2
254-26	.80	<.06	.15	1.2
262-159	.32	<.06	<.10	2.0
262-85	.32	<.06	<.10	3.7
262-69	2.9	<.06	2.1	2.8
262-41	1.4	<.06	<.10	1.0
267-155	.19	<.06	<.10	2.0
267-136	1.8	<.06	<.10	1.0
267-111	3.7	<.06	<.10	1.0
267-88	3.5	<.06	7.6	1.2
271-165	<.06	<.06	<.10	1.0
271-141	.56	<.06	<.10	2.0
271-85	.98	<.06	<.10	2.0
271-41	.07	<.06	<.10	1.0
279-86	.42	<.06	<.10	1.0
279-61	.25	<.06	<.10	<1.0
282-123	.28	<.06	<.10	3.1

Table 15.--Water sample analyses, Cape Cod, Massachusetts July-August, 1983--Continued.

[A dash indicates no analysis was available]

Well number	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	NH_4^+ (mg/L as N)	Dissolved organic carbon (mg/L)
FSW				
282-94	0.78	<0.06	<0.10	4.0
282-70	1.36	<.06	<.10	4.2
282-49	<.06	<.06	<.10	1.9
288-97	3.8	<.06	2.3	2.2
290-91	2.1	<.06	<.10	1.0
294-89	1.4	<.06	<.10	3.1
299-20	3.1	<.06	<.10	2.0
300-30	14	<.06	5.3	3.5
300-10	.26	<.06	<.10	1.0
347-67	<.06	<.06	2.1	4.0
SDW				
305-32	3.8	<.06	<.10	3.0
313-60	.90	<.06	<.10	1.0

Table 15.--Water sample analyses, Cape Cod, Massachusetts July-August, 1983--Continued.

[A dash indicates no analysis was available]

Well number	DSEA counts (CFU/mL)	NA counts (CFU/mL)	MPN (coliform)
FSW			
166-67	7	540	0*
167-55	1,490	370	0
173-69	35	--	--
182-69	5	--	0
194-57	24	--	0
214-60	11	--	0
230-48	1,570	540	0
231-57	332	--	--
232-58	910	280	0
234-99	26	--	0
235-94	27	--	0
236-106	219	90	0
237-88	90	80	0
238-106	259	65	0
239-64	800	288	0
242-77	27	--	0
243-45	--	--	--
244-90	640	176	0
245-25	7	--	0
246-35	17	11	0
247-70	46	--	--

*0 = negative, *+ = positive*, D = doubtful

Table 15.--Water sample analyses, Cape Cod, Massachusetts July-August, 1983--Continued.

[A dash indicates no analysis was available]

Well number	DSEA counts (CFU/mL)	NA counts (CFU/mL)	MPN (coliform)
FSW			
254-216	1,500	550	0
254-168	1,130	390	0
254-140	1,500	700	0
254-107	5,670	3,000	0
254-72	22,680	22,580	0*
254-54	5,100	3,480	0
254-26	470	240	0
262-159	5,600	--	--
262-85	7,100	1,950	+*
262-69	570	318	0
262-41	15	5	0
267-155	1,020	560	0
267-136	1,150	68	0
267-111	40	16	0
267-88	180	77	0
271-165	3,400	1,420	0
271-141	11	12	0
271-85	1,150	50	0
271-41	10	6	0
279-86	80	69	0
279-61	50	13	0

*0 = negative, *+ = positive*, D = doubtful

Table 15.--Water sample analyses, Cape Cod, Massachusetts July-August, 1983--Continued.

[A dash indicates no analysis was available]

Well number	DSEA counts (CFU/mL)	NA counts (CFU/mL)	MPN (coliform)
FSW			
282-123	120	80	0
282-94	4,500	2,700	0
282-70	1,060	490	0
282-49	--	--	0
288-97	1,400	1,140	0
290-91	11	5	0
294-89	18,140	2,390	0
299-20	80	--	0
300-30	300	57	0
300-10	17	13	0
347-67	160	70	0
SDW			
305-32	--	350	0
313-60	50	17	0

*0 = negative, *+ = positive*, D = doubtful

Analysis of Inorganic Constituents

Specific conductance was measured with a conductivity meter, and dissolved oxygen was assayed with a Yellow Springs Instrument Company dissolved oxygen meter.[2] Temperature was measured with an ordinary mercury thermometer and pH was measured with a glass electrode pH meter.

Nitrate analysis was done at a laboratory on site using the colorimetric cadium-reduction method (American Public Health Association, 1981). Nitrate is reduced to nitrite in the presence of cadmium. Nitrite is then diazotized with sulfanilamide, and coupled with N-(1 naphthl)-ethylenediamine, to form a highly colored dye. The concentration of this dye is measured colorimetrically at 540 nm. Correction for any nitrite present in the sample is made by analyzing without the reduction step.

Water samples were analyzed for ammonium by colorimetric analysis. The method was adapted from the automated method used by the U.S. Geological Survey National Water Quality Laboratory, Arvada, Colorado (Skougstad and others, 1979). Inorganic ammonium reacts with sodium salicylate and sodium nitroferricyanide in an alkaline medium to form a colored compound, the absorbance of which is directly proportional to the ammonium concentration.

Samples were analyzed for phosphate by the same laboratory by an automated colorimetric method (Skougstad and others, 1979). Orthophosphate ion reacts with ammonium molybdate in acidic solution to form phosphomolybdic acid. When phosphomolybdic acid is reduced with ascorbic acid, an intensely colored blue complex is formed that is measured by a colorimeter at 660 nm.

Dissolved Organic Carbon

Dissolved organic carbon (DOC) analyses were done by Huffman Laboratory (Arvada, Colorado) using a 5020 Coulometrics total carbon apparatus. After filtration through a Selas 0.45 µm silver membrane filter, the sample is acidified to pH 2 with hydrochloric acid, and purged to remove inorganic carbon. A 0.2-mL aliquot of sample is injected into the carbon analyzer where carbon is completely oxidized to carbon dioxide in an oxygen atmosphere at 1,000°C. The combustion gases are swept into a Coulometrics carbon dioxide coulometer, and the carbon dioxide in the gas stream is measured.

Enumeration of Viable Cells

Plate counts were made on nutrient agar and dilute soil-extract agar by the pour-plate method (Ghiorse and Balkwill, 1981). Dilute soil-extract agar (DSEA) is prepared by autoclaving 500 g of sandy-loam surface soil in 500 mL of distilled water for one hour at 121°C. The resulting extract is filtered. The filtrate is diluted tenfold with distilled water, and is amended with 1.5 percent Bactoagar. Media are sterilized by autoclaving at 121°C for 20 minutes.

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[2]The use of trade names is for descriptive purposes only, and does not constitute endorsement by the U.S. Geological Survey.

Because of the large number of samples and time limitations, plate counts were not replicated, but, in every case three serial dilutions were done, and there seemed to be satisfactory agreement between dilutions. Volumes of 1-mL, 0.1-mL, and 0.01-mL water samples were added to pour plates of Difco nutrient agar and DSEA agar. These plates were incubated for one week at 22 to 24°C. After the incubation period, the number of colony-forming units per milliliter (CFU/mL) were counted. Coliform bacteria were determined with the MPN (most probable number) presumptive coliform test (American Public Health Association, 1981) using three tubes of lauryl tryptose broth for each dilution. Confirmational tests were not performed.

EVIDENCE OF MICROBIAL PROCESSES

Temperature, pH, and Specific Conductance

The temperature of samples of ground water collected from wells ranged from 9.0 to 13.5°C, and pH ranged from 5.3 to 7.1 pH units. Uncontaminated ground water has a specific conductance of 40-80 µmhos, whereas the treated sewage has a specific conductance of 400 µmhos. Specific conductance has been used previously by LeBlanc (1982) to delineate the plume. Conductivity is a useful indicator of the extent of the plume because the specific conductance of the treated sewage is much higher than that of uncontaminated ground water.

A vertical section of the specific conductance data along the axis of the plume is shown on figure 42. The area of conductivity greater than 200 µmhos extends at least 11,000 feet downgradient and is 85 feet thick 3,000 feet downgradient from the sand beds. The area of highest specific conductance is immediately adjacent to the sand beds.

Dissolved Oxygen

The distribution of dissolved oxygen in ground water is shown in figure 43. Data from 1979 (LeBlanc, 1982) were used because the dissolved oxygen data for 1983 are questionable due to mechanical problems with the dissolved oxygen meter. Within 5,000 feet of the sand beds, dissolved oxygen is present in a thin boundary zone (about 25 feet thick) along the top of the plume. Dissolved oxygen in this zone is derived from recharge through the sandy soils that are low in organic matter content (LeBlanc, 1982). Dissolved oxygen is not present in the center of the plume within 5,000 feet of the sand beds.

At about 6,000 feet downgradient from the sand beds, dissolved oxygen is found in the center of the plume. The presence of dissolved oxygen at this location is caused by mixing of the contaminated and uncontaminated ground water, and possibly by insufficient oxygen demand. No dissolved oxygen was found below the plume in the deeper part of the aquifer.

Nitrate, Ammonia, and Phosphate

Domestic sewage contains many organic and inorganic nitrogen compounds derived from human wastes and food wastes. Nitrogen in organic compounds is readily converted to ammonium by bacterial processes. The inorganic species are predominantly ammonium and nitrate. The nitrate concentration in the effluent is 12 mg/L and is about twice as high as the ammonium concentration in the effluent (6.4 mg/L).

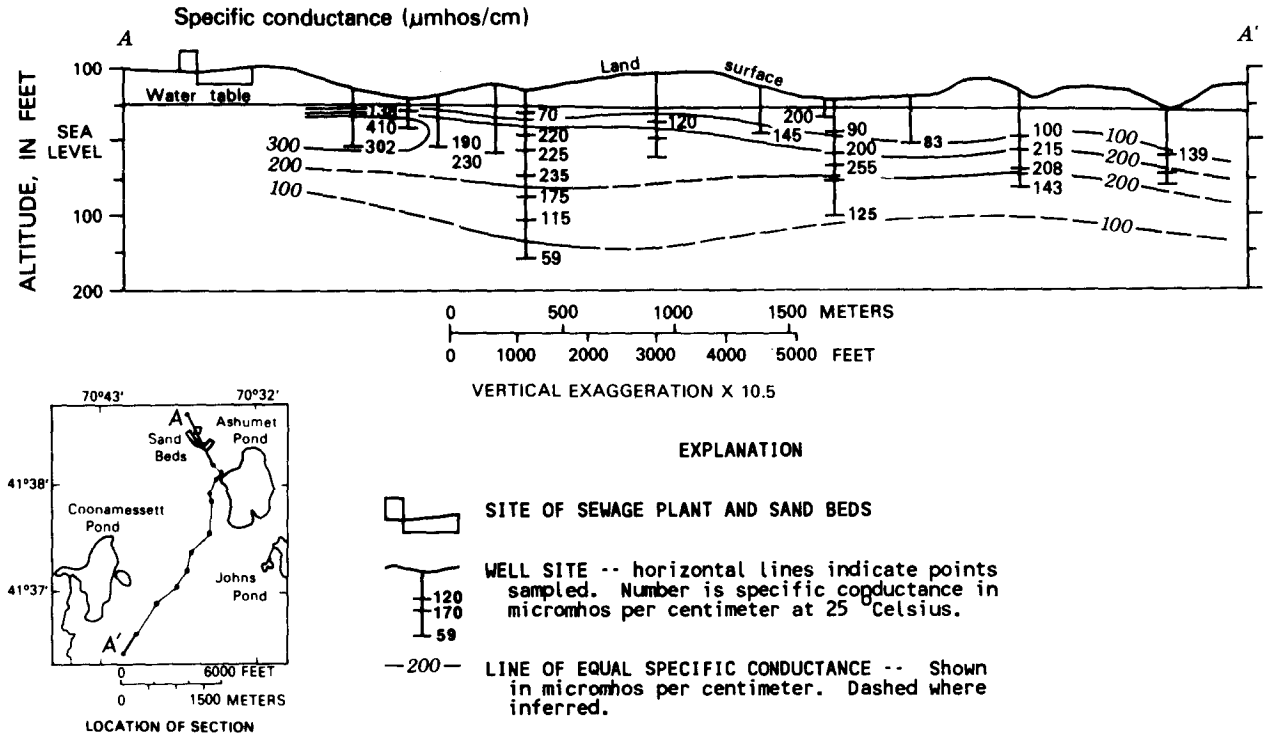

Figure 42.--Vertical distribution of specific conductance in ground water, July-August 1983.

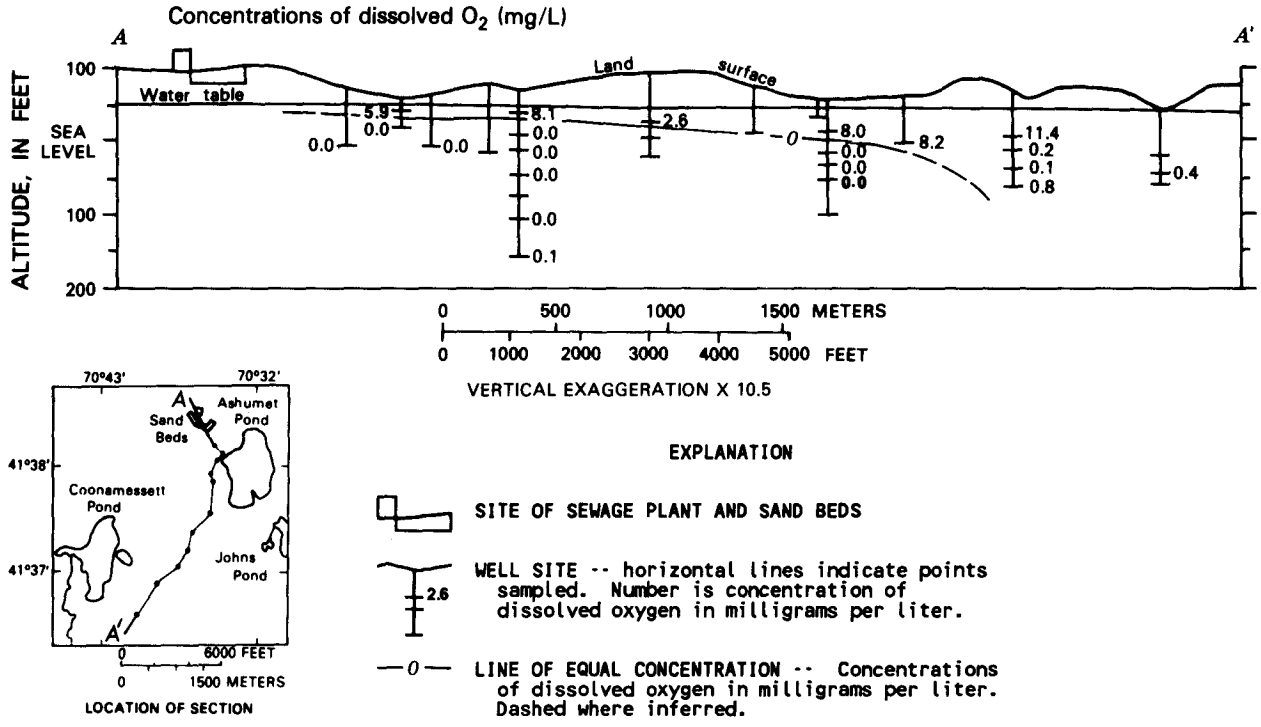

Figure 43.--Vertical distribution of dissolved oxygen in ground water, May 1978 through May 1979 (from LeBlanc, 1982).

As can be seen in figure 44, the ammonium plume (indicated by the area with concentrations greater than 2 mg/L) extends 8,000 feet downgradient of the sand beds. The highest ammonium concentrations are 1,000 to 3,000 feet from the sand beds. The area where nitrate concentrations are greater than 1 mg/L extends at least 11,000 feet from the sand beds. Higher nitrate concentrations (greater than 2 mg/L) are found about 6,000 feet downgradient. The distribution of nitrate in 1983 is similar to the distribution in 1978-79 (LeBlanc, 1982).

Although the nitrate concentration in the effluent is 12 mg/L, the concentration of nitrate in the center of the plume is only 2.9 mg/L. Studies have shown that nitrate is readily reduced in anaerobic soils and sediment (Smith and others, 1982). Two types of bacterially mediated nitrate reduction can occur: (1) Nitrate assimilation, which involves uptake of nitrate by the cells, reduction to ammonium and incorporation into microbial cellular material; and (2) nitrate respiration, which is a dissimilatory process whereby nitrate functions as the terminal electron acceptor in the absence of oxygen. Nitrate respiration consists of two possible mechanisms: (1) Denitrification, which is the reduction of nitrate to molecular nitrogen; and (2) dissimilatory nitrate reduction, which is the reduction of nitrate to ammonium. The loss of nitrate as the effluent enters the aquifer may result from denitrification because the product is a gaseous species. Also, it is evident from figure 44 that the zone of high ammonium concentration is generally coincident with the zone of low nitrate concentration; therefore, dissimilatory nitrate reduction, in which ammonium is being produced, is another possible nitrate sink.

Within 6,000 feet of the sand beds, the predominant nitrogen species in the plume is ammonium. At 6,000 feet from the source of contamination, the predominant chemical species of inorganic nitrogen changes from ammonium to nitrate. LeBlanc (1982) suggested that the observed ammonium-nitrate distributions could be explained by nitrification. The process of nitrification is the oxidation of ammonium to nitrate by the nitrifying bacteria Nitrosomonas and Nitrobacter. These organisms generally grow autotrophically, although it has been shown that some species of Nitrobacter grow heterotrophically (Kalthoff and others, 1979; Smith and others, 1982; Bock, 1976). Autotrophic organisms are not inhibited by the low carbon content of ground water, and previous studies have shown that nitrifiers are indigenous to ground water (Behnke, 1975). Dissolved oxygen is a requirement for nitrifying bacteria. At about 6,000 feet downgradient from the sand beds, dissolved oxygen is present in the center of the plume; corresponding decreases in ammonium and increases in nitrate in this area indicate that nitrification may be occurring here. Nitrate, but no ammonium is present beyond 8,000 feet from the sand beds.

Most phosphorus in secondarily treated sewage is in the form of orthophosphate. The average concentration of orthophosphate in treated sewage is 7 mg/L: yet, orthophosphate has not moved more than 1,000 feet from the sand beds (fig. 44). One explanation for this is that phosphate may precipitate as the minerals hydroxyapatite ($Ksp=10^{-57.8}$) and fluoroapatite ($Ksp=10^{-60.4}$). Calcium (0.4 to 17.0 mg/L) and fluoride (0.01 to 0.27 mg/L) are both present in the plume; thus, phosphate may be removed near the infiltration beds. Alternatively, phosphate may be removed by bacterial uptake.

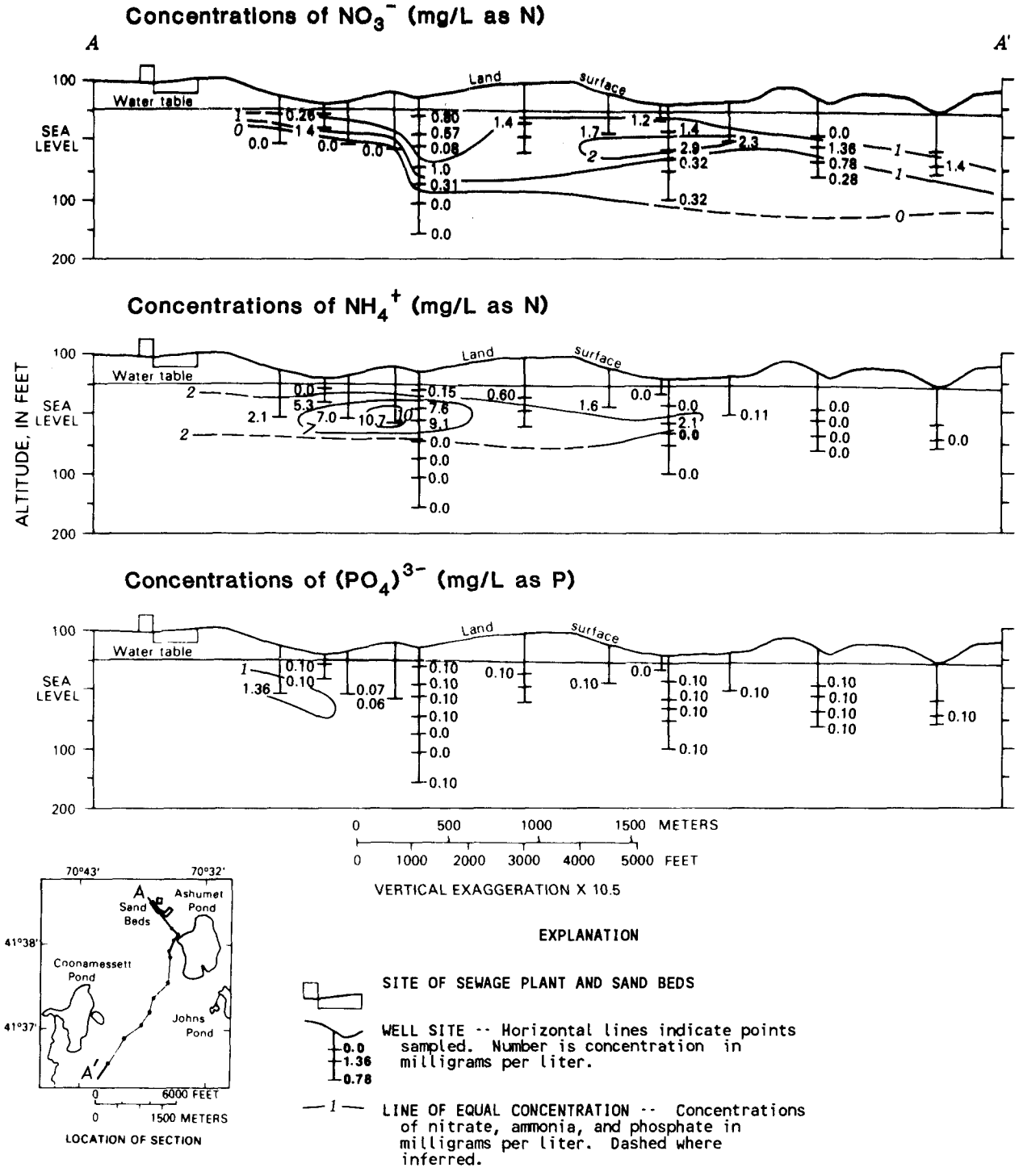

Figure 44.--Vertical distribution of nitrate, ammonia, and phosphate in ground water, July-August, 1983.

Plate Counts

The counts on nutrient agar and dilute soil-extract agar (DSEA) give similar spatial profiles of the plume (fig. 45). The plate counts show areas of elevated bacterial counts (greater than 4,000 CFU/mL for DSEA and greater than 2,000 CFU/mL for nutrient agar) that extend at least 11,000 feet downgradient from the sand beds. These profiles indicate a positive response of the microbial population to the chemical plume as delineated by conductivity, nitrate, and DOC.

Nutrient agar is a rich organic medium while DSEA has lower levels of organic nutrients. In general, DSEA medium yielded higher cell counts and smaller colonies than nutrient agar. Similar results have been found in other aquifer systems (Ghiorse and Balkwill, 1981; Wilson and others, 1983). Wilson and his coauthors concluded that DSEA medium was preferred over rich organic media because higher numbers of plate count bacteria were obtained with DSEA. In a ground-water system where organisms are adapted to oligotrophic conditions, it is not surprising that DSEA was the preferred medium.

It is important to understand that plate counts are not total cell counts. Because of the existence of aggregates of cells, several cells may produce just one colony. Also, some microorganisms from the subsurface may not grow on nutrient agar or DSEA.

Dissolved Organic Carbon

The shape and extent of the dissolved organic carbon (DOC) plume is similar to the conductance plume. Two locations of high DOC were found (fig. 45). One area is in the immediate vicinity of the sand beds; the other area is 8,000 to 9,000 feet downgradient from the sand beds, where 50 percent of the DOC is in the form of nonbiodegradable detergents (Thurman and others, 1984). The detergent plume does not correlate with the plate counts, and it is not biodegradable.

The DOC in the effluent is 12 mg/L; however, within 1,000 feet of the sand beds, the DOC is quickly depleted to 1-2 mg/L. Microbes in the subsurface are likely a major mechanism for the removal of organic matter. The relationships between depth, distance from the sand beds, DOC, and bacterial populations for the well clusters FSW 254, FSW 262, and FSW 282 are shown in table 16. In general, depth profiles of bacterial plate counts display a pattern similar to depth profiles of DOC. In addition, a strong similarity exists between DOC distribution and bacterial counts along a horizontal transect away from the sand beds (fig. 45), which suggests that microbial processes are involved in the removal of organic matter in this ground-water system. A corresponding increase in alkalinity within 1,000 to 2,000 feet of the infiltration beds also suggests that microbial processes are mineralizing organic carbon to carbon dioxide (Thurman and others, 1984).

Coliforms

Coliform bacteria (specifically fecal coliform) inhabit the intestines of warm-blooded animals, and are typically present in sewage; therefore, these bacteria serve as indicators of potential fecal pollution. Previous studies have shown that coliforms are not transported in ground water. Vecchioli and others (1972) found that coliforms generally were not transported very far from an injection well because of filtration. A study on the movement of coliforms from a septic tank effluent (Reneau and Pettry, 1975) indicated that total and fecal coliforms decreased significantly with horizontal

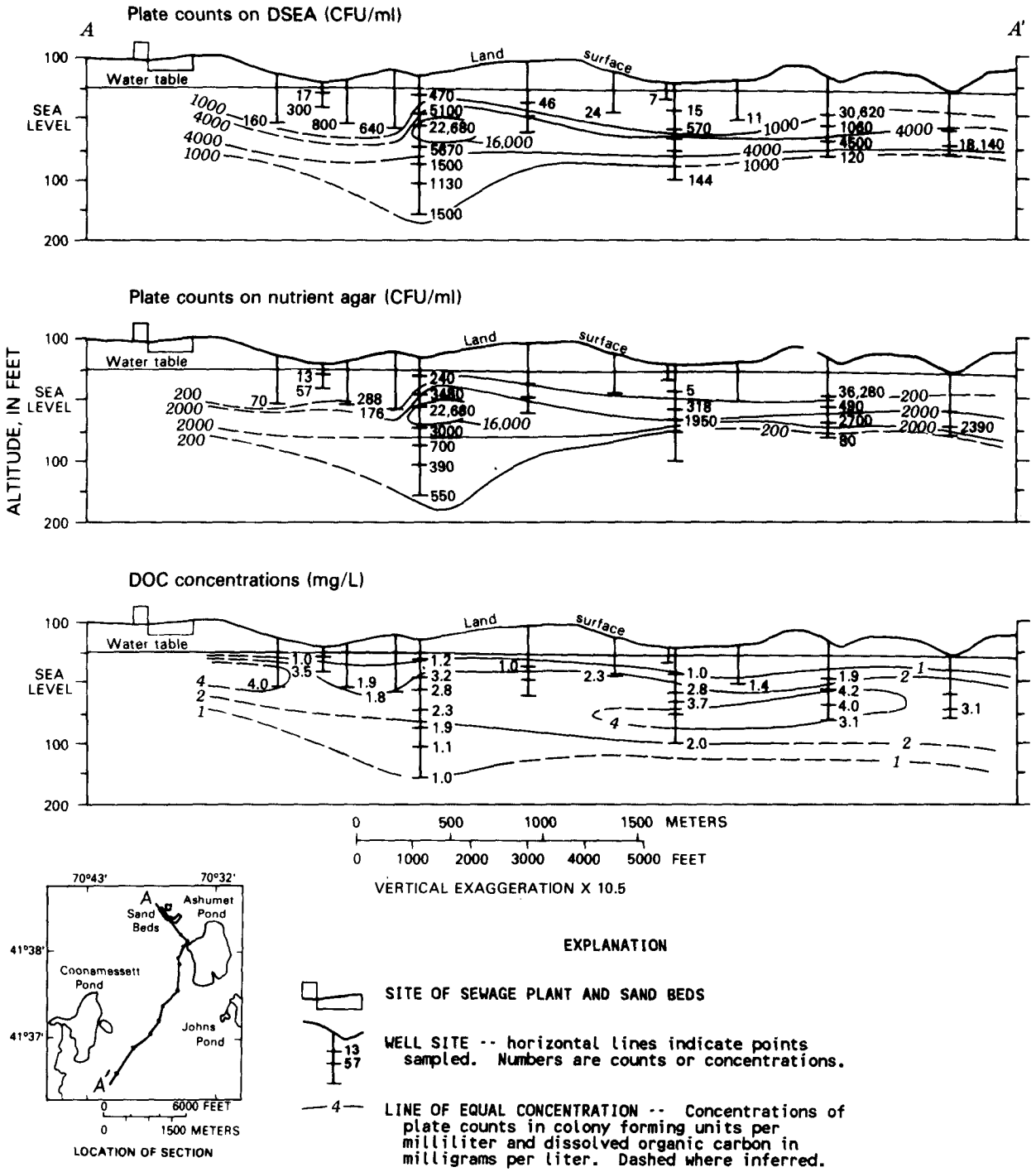

Figure 45.--Vertical distribution of plate counts and dissolved organic carbon in ground water, July-August 1983.

Table 16--Dissolved organic carbon concentrations and bacterial counts in ground water, July-August 1983.

[a dash indicates no analysis was available]

Well number	Depth from surface	Distance from beds (ft)	DOC (mg/L)	DSEA plate counts (CFU/mL)
FSW 254-216	216	3000	1.0	1500
254-168	168	3000	1.1	1130
254-140	140	3000	1.9	1500
254-107	107	3000	2.3	5670
254-72	72	3000	2.8	22680
254-54	54	3000	3.2	5100
254-26	26	3000	1.2	470
262-159	159	7000	2.0	144
262-109	109	7000	4.0	5600
262-85	85	7000	3.7	7100
262-69	69	7000	2.8	570
262-41	41	7000	1.0	15
282-123	123	9000	3.1	120
282-94	94	9000	4.0	4500
282-70	70	9000	4.2	1060
282-49	49	9000	1.9	----

distance and depth. More recently, however, Rahe and others (1978) found movement rates of 1500 cm/hour for Escherichia coli in soils under conditions of saturated flow. The same study indicated that Escherichia coli survived for at least 96 hours in the soils, and could be used as tracers for subsurface water flow.

In this study, no coliform bacteria could be detected, except in wells FSW 254-107 and FSW 262-85 (see figure 41 for location of wells). Neither of these wells is in the vicinity of the sand beds; therefore, coliforms from the sewage do not appear to be transported. The reason that coliform bacteria were detected in two of the wells is unknown, and these wells should be resampled next field season.

SUMMARY AND CONCLUSIONS

During the summer of 1983, a broad reconnaissance of microbial numbers and chemical composition in the plume of sewage-contaminated ground water was conducted at Otis Air Base, Cape Cod, Massachusetts. Water analyses reveal that microbial processes may play a major role in determining the fate of contaminants in the ground water. Smith and Duff (1984) have conducted a study of denitrification in the plume. Other pertinent processes, such as nitrification and bacterial uptake of organics, should be investigated.

The major conclusions of this study are:

1. Nitrate is present in high concentrations in the sewage effluent, but is depleted in the zone of contamination within 1000 feet of the sand beds. This implies that a nitrate sink exists within the aquifer which may be a result of denitrification, nitrogen assimilation to organic nitrogen, and reduction of nitrate to ammonium.

2. Ammonium is present in high concentrations a short distance downgradient of the sand beds and is depleted or absent further downgradient where nitrate levels are elevated and oxygen is present. This implies that ammonium is being oxidized to nitrate, perhaps by microbial nitrification.

3. Colony counts on nutrient agar and dilute soil-extract agar give comparable spatial distributions.

4. Dilute soil-extract agar is a better medium than nutrient agar for bacterial enumeration from subsurface environments.

5. The removal of dissolved organic carbon in the plume appears to be due, in part, to microbial degradation.

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CHAPTER F

The previous paper by Ceazan, Updegraff, and Thurman presented evidence that bacteria are degrading and transforming contaminants in the plume. In this paper, the size, distribution, and general level of activity of the bacterial population is evaluated at several locations in the aquifer. This information is needed to determine the rates and conditions under which these microbes degrade and transform contaminants.

MICROBIAL DISTRIBUTION AND HETEROTROPHIC UPTAKE IN A SEWAGE PLUME

by Ronald W. Harvey, Richard L. Smith, and Leah George

ABSTRACT

The bacterial abundance, distribution, and heterotrophic uptake in ground water contaminated by treated sewage at Otis Air Base, Massachusetts, was determined from ground water and sediment-core samples. Numbers of free-living bacteria in contaminated ground water declined steadily with increasing distance from the infiltration sand beds where the treated sewage recharges the aquifer. The numbers decline from $1.94 \pm 0.20 \times 10^6$ mL^{-1} (per milliliter) at 0.21 km (kilometers) from the beds to $0.25 \pm 0.02 \times 10^6$ mL^{-1} at 0.97 km from the beds. Bacterial abundances in ground water sampled from a cluster of wells located 0.31 km from the beds correlated strongly with specific conductance and increased sharply from $0.04 \pm 0.003 \times 10^6$ mL^{-1} at depth of 6 m (meters) to $1.58 \pm 0.12 \times 10^6$ mL^{-1} at a depth of 14 m, then declined at depths of 20 m and 31 m to $1.29 \pm 0.12 \times 10^6$ mL^{-1} and $0.96 \pm 0.12 \times 10^6$ mL^{-1}, respectively. A majority of the bacteria in contaminated and uncontaminated zones of the aquifer were bound to surfaces of particulates with diameters of less than 60 micrometers. Glucose-uptake rates, assayed at in situ and 5 uM (micromolar) concentrations, declined steadily in ground water sampled along a transect that extends from 0.64 to 1.80 km. A preparative wet-sieving technique for use in processing core samples for bacterial enumeration was found to yield higher counts than a previously used preparative settling technique.

INTRODUCTION

Microbial degradation of organic contaminants in ground water has been a subject of considerable recent interest, but little is known about sizes, distributions, and activities of bacterial populations in freshwater aquifers. Much of our present information derives from studies performed on well water samples. However, a majority of the bacterial population in the saturated zone of the terrestrial subsurface may be associated

with solid surfaces and would not be accounted for in such investigations. Also, few definitive studies have dealt with the response of ground-water bacteria to significant organic contamination.

A study was made to examine partitioning of aquifer bacteria between solid surfaces and solution. This information may be useful in predicting the fate of organic contaminants in ground water. Another objective was to examine the relationship between bacterial numbers and uptake rates for glucose in contaminated ground water as a function of distance from the source of contamination. The hypothesis is that localized organic contamination of oligotrophic ground water should result in a zone in which size and activity of the bacterial population is dependent, in part, upon concentrations of readily-degraded organics and, hence, distance from the source of contamination. In this report, we describe the effect of ground-water contamination upon bacterial abundances, distribution, solid/solution partitioning, and glucose uptake rates in a plume formed by land disposal of treated sewage on Cape Cod, Massachusetts[1]. We also evaluate a preparative technique used in conjunction with acridine-orange epifluorescence to enumerate particle-bound bacteria in aquifer core material.

The authors thank D.R. LeBlanc, W. Nichols, and others with the New England District for their help and technical assistance.

EXPERIMENTAL PROCEDURES

Collection of Samples

Water samples for microbial assays were obtained from a network of screened, PVC (polyvinyl-chloride) observation wells that are 3.8 or 5.0 cm in diameter. The wells, which have PVC screens with slots 250 µm wide, are located along the path of the plume (fig.46). At three sites located 0.21, 0.31 and 2.9 km from the infiltration beds, clusters of wells screened at a different depth were sampled to obtain depth profiles of the plume. A stainless-steel submersible pump (model SP-81, Keck Geophysical Instruments, Inc. Okemos, Michigan) connected to Teflon tubing was used to sample ground water from the wells. Samples were taken in sterile glass and plastic bottles after three to five well volumes had been pumped and specific conductance and pH had stabilized. Plastic gloves were worn during the sampling procedure to reduce sample contamination from skin-associated flora. Samples used for total bacterial counts were fixed immediately with glutaraldehyde (0.2 percent weight per volume final concentration); all samples were kept on ice during transport and stored at 4°C in the laboratory. Where possible, samples were processed within 48 hours.

Six core samples of aquifer sediments were collected at depths ranging from 12 to 32 m below land surface at distances of 0.21 and 2.93 km from the source of contamination. This was

[1]A description of the site, including its geologic and hydrologic setting, is given in chapter A of this volume.

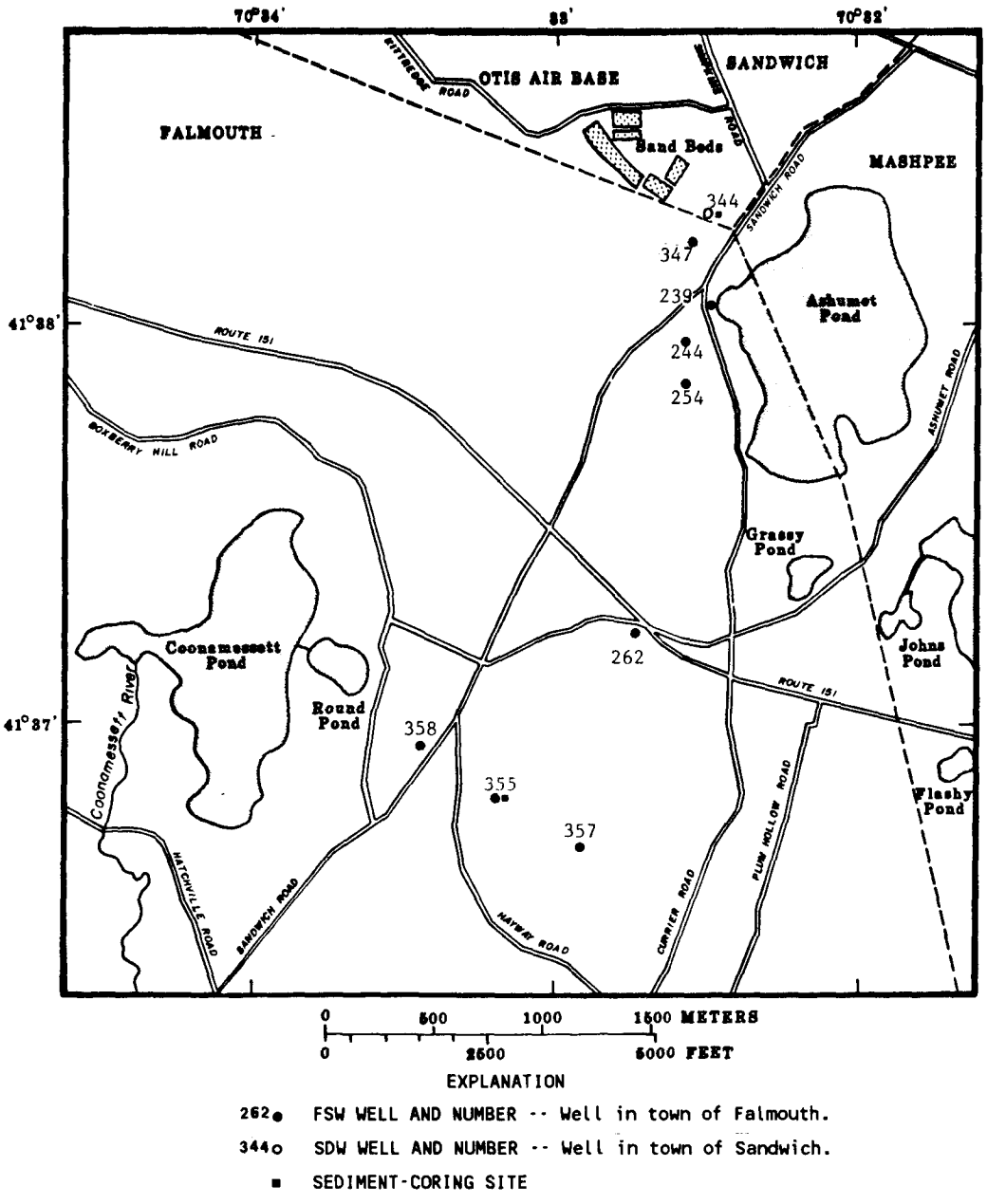

Figure 46.--Study area and data-collection sites.

accomplished by means of a split-spoon sampling device lowered down the hollow barrel of an auger drilling rig and driven 0.3 to 0.6 m below the bottom of the augers. Approximately 1 kg of solid aquifer material was stored in sterile 500 mL bottles and kept on ice until processed. Data obtained from core samples were compared to that obtained from ground water sampled from adjacent wells at equivalent depths.

Enumeration of Bacteria

Free-living bacteria in well-water samples were enumerated using an acridine-orange direct counting (AODC) procedure (Hobbie and others, 1977). Since quantities of abiotic suspended particulates in sampled well water were low, between 1 and 40 ml of undiluted sample could be used for each acridine-orange preparation. The amount of sample used depended upon bacterial abundance. Particle-bound bacteria, which constituted less than 5 percent of the total sample population, were not enumerated. A Leitz Dialux 20 microscope, fitted for epifluorescence (HBO 100w Hg lamp, H-2 barrier/exciter filter system with K480 quenching filter, 100x (1.32NA) NPL Fluotar iris objective) was used to enumerate bacteria at a total magnification of 1000x.

Enumerations of adherent bacteria in core samples were made for four particle-size fractions. Core subsamples of approximately 100 g were weighed and aseptically wet-sieved through a sequential series of mesh filters having progressively smaller mesh openings: 105µm (polypropylene), 60µm (nylon), and 20um (nylon) (Spectrum Medical Industries, Inc.). Sieved particulates were collected in sterile 400 mL Pyrex beakers containing approximately 100mL of water. Particles retained on each mesh filter were voided of remaining interstitial water using a gentle rinse and collected in 250 mL polypropylene bottles, which were then filled with filter-sterilized water. All preparative operations were performed using a laminar flow hood (greater than 99.9 % efficiency at 0.3 µm), surgical gloves, sterile apparatus, and filter-sterilized (0.2 µm), Milli-Q purified water (18 megaohm resistivity). Levels of contamination introduced in sample processing were accounted for with a control blank, which was prepared by NaOH-washing (0.1N) of a subsample prior to the preparative procedure.

Well-stirred, 250-mL suspensions of size-fractionated particulates in the three smaller size fractions were sampled by Eppendorf pipet and assayed for solids content and population density of adherent bacteria (Rublee and Dornseif, 1978). For the largest size fractions, which consisted of sand grains with diameters greater than 105 µm, adherent bacteria were enumerated on individual grains embedded in 0.1 percent agar under 500x magnification. Calculated concentrations of adherent bacteria were expressed both as numbers per unit dry weight of size-fractionated and total sediment samples and as numbers per unit volume of aquifer material. Porosities used in these calculations were estimated from amounts of water which could be added to dry, weighed core material occupying a known volume. The wet-sieving procedure was compared to a preparative settling technique described in an earlier subsurface microbial study

(Wilson and others, 1983). In the latter study, a 2-minute settling procedure was used to void core-material suspensions of the largest sand grains, which interfere in the enumeration procedure.

Tritiated-glucose uptake by ground-water bacteria was measured in four well water samples obtained along an 0.6 km-long transect within the plume. Four 50 mL aliquots of each sample were placed in plastic 50 mL syringes which were then sealed with rubber-stoppered injection hubs. One syringe was injected with NaOH (0.1N final concentration) and served as a killed control. A second syringe was amended with glucose (5 µM final concentration). All syringes were injected with similar quantities of ^{3}H-glucose (New England Nuclear, 14.5 Ci/mmole, 3.6 uCi total), shaken, and incubated at 12°C. The third and fourth syringes were assumed to contain near in-situ concentrations of glucose, since final concentrations of added ^{3}H-glucose were 5 nM. A 10 mL subsample from each syringe was filtered through a Gelman metricel filter (0.2 µm pore size, 25 mm diameter) after 0, 5, 10, 22, and 48 hours of incubation. The filters were then clarified in 10 mL of aquasol, dark-adapted, and assayed for ^{3}H activity by liquid scintillation counting using the internal standard addition (^{3}H-toluene) method for quench-factor correction.

MICROBIAL DISTRIBUTION AND HETEROTROPHIC UPTAKE

Microbial Distribution

Concentrations of ground-water bacteria along the path of the plume are depicted as a function of distance from the infiltration beds in figure 47. Bacterial numbers in ground water sampled from the middle of the plume declined sharply from $1.94\pm0.20\times10^{6}$ mL^{-1} at 0.21 km from the beds to $0.25\pm0.02\times10^{6}$ mL^{-1} at 0.93 km. However, bacterial counts in contaminated ground water from the three more distant sampling locations ($0.21\pm0.02\times10^{6}$ mL^{-1}, average) were significantly higher than counts for uncontaminated ground water ($0.04\pm0.005\times10^{6}$ mL^{-1}) sampled in the same region but from a shallower depth.

Bacterial numbers in the well water as a function of the depth of the well screen at two sites located 0.21 and 0.31 Km from the beds are shown in figure 48. At both sites, bacterial abundance was lowest in samples taken from the shallowest well, increased at the next depth, and decreased in the two deepest wells. At the site located 0.31 km from the beds, bacterial concentrations increased from $0.04\pm0.003\times10^{6}$ mL^{-1} at 6m below land surface to $1.58\pm0.12\times10^{6}$ mL^{-1} at 14 m, a 40-fold increase within 8 m. Specific conductance increased over four fold in these same samples from 68 µmhos to 300 µmhos. Numbers of free-living bacteria at both sites correlated strongly with specific conductance. The coefficients of determinations (r^{2}) at the two sites were 0.91 and 0.96 ($p\leq0.05$).

Counts of surface-attached bacteria per unit volume of aquifer for six core samples taken in the saturated zone are listed in table 17. Counts of free-living bacteria in water collected from wells screened at the same depth as the cores are

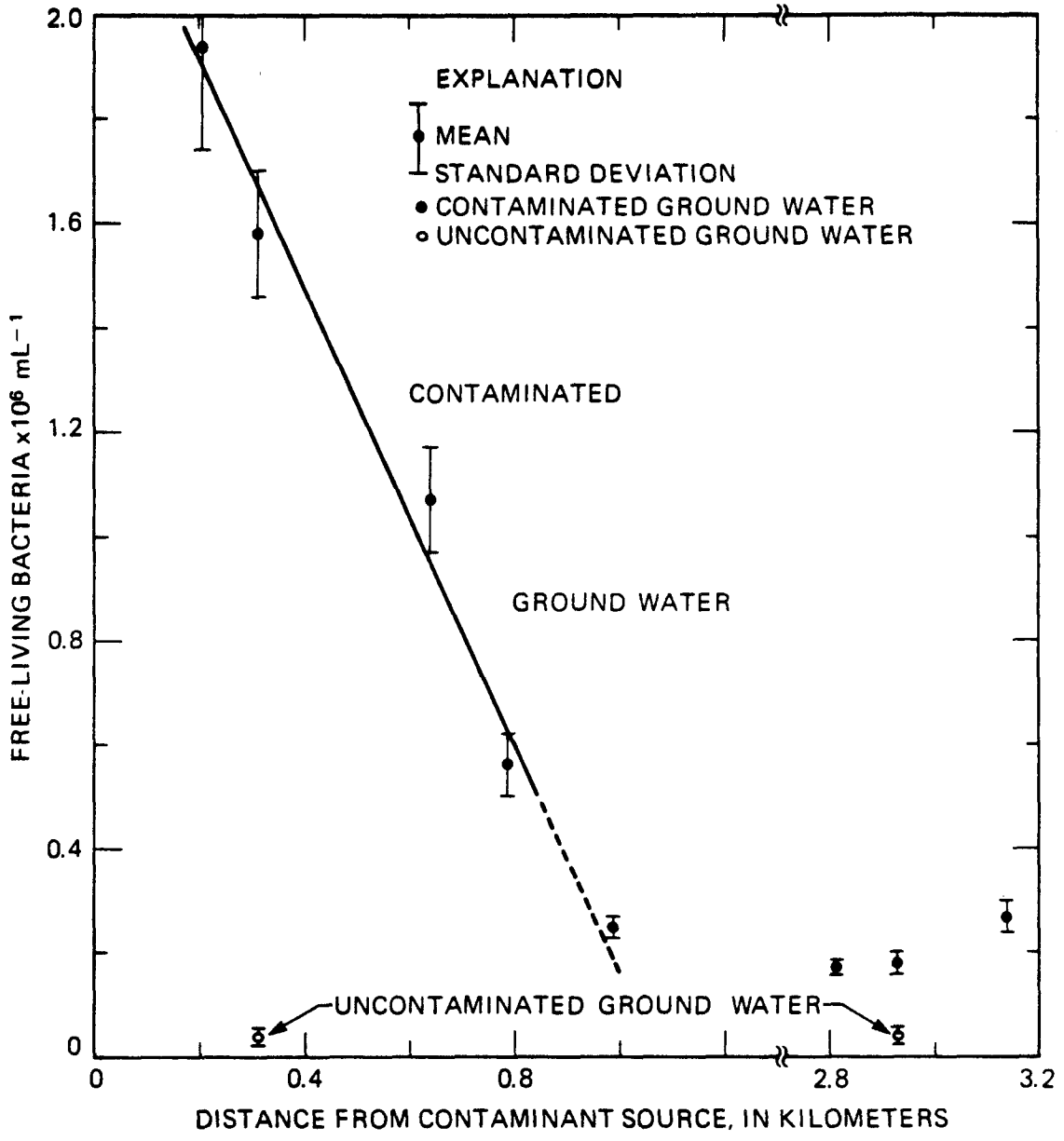

Figure 47. Numbers of free-living bacteria in ground water versus distance from sewage disposal beds. Straight line indicates the best fit using linear regression (r^2=0.98 at $p \leq 005$).

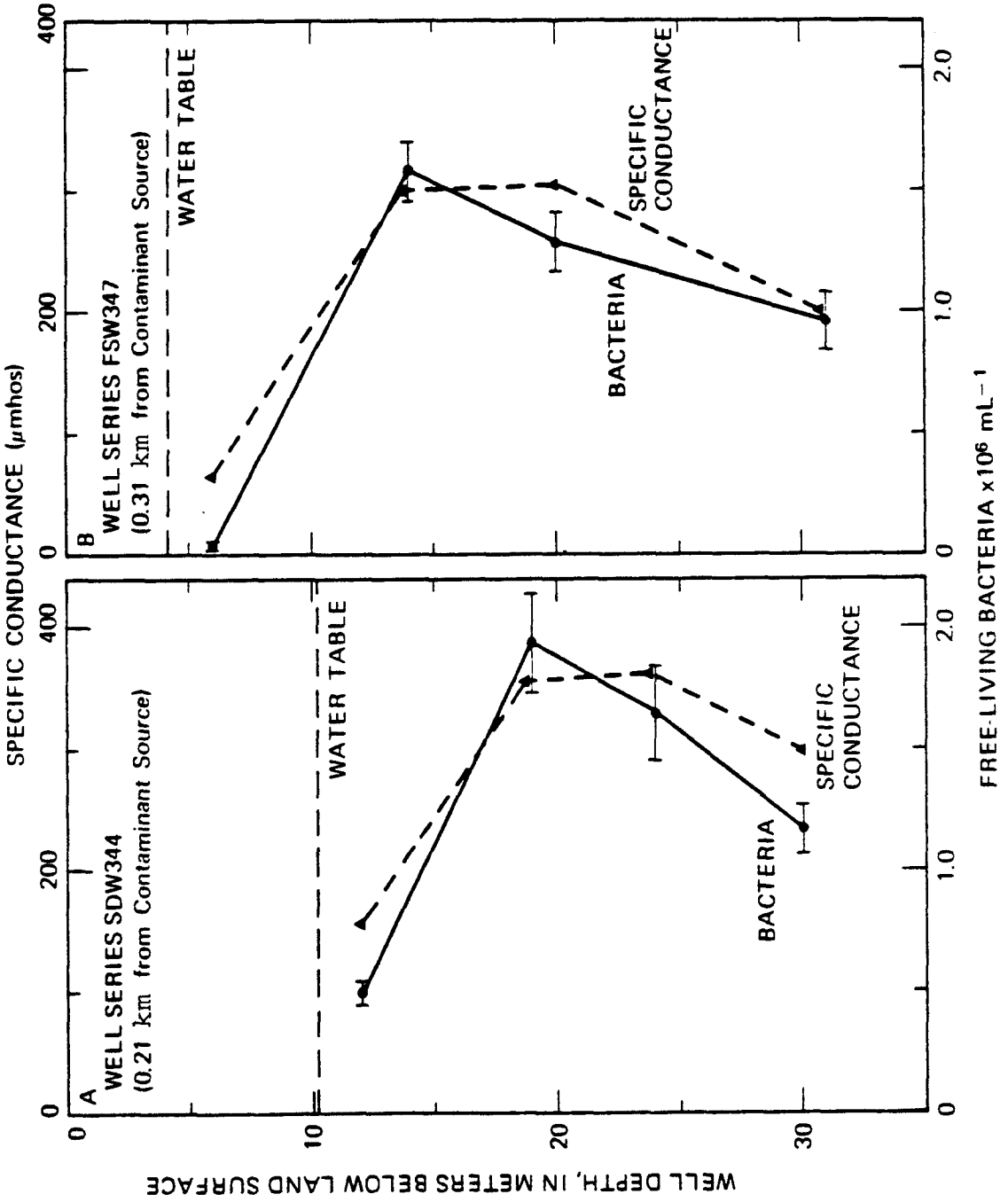

Figure 48. Numbers of free-living bacteria and specific conductance versus depth below land surface for two clusters of observation wells located 0.21 km (A) and 0.31 km (B) from the sewage disposal area. Difference in water-table depth between the two well clusters is due to topographical variation.

Table 17. Bacterial numbers and distribution in contaminated and uncontaminated zones

Distance to contaminant source (km)	Sample depth (m)	Ground water specific conductance (μmhos)	Bacteria (per cm^3 aquifer material)		
			Free-living[1]	Particle-bound[2]	(percent bound)
0.21	12	158	$1.87\pm0.19\times10^5$	$1.84\pm0.22\times10^7$	(98.9)
0.21	19	355	$6.42\pm0.66\times10^5$	$1.94\pm0.29\times10^7$	(96.8)
0.21	25	365	$5.38\pm0.62\times10^5$	$4.04\pm0.38\times10^7$	(98.7)
0.21	31	300	$3.98\pm0.34\times10^5$	$2.29\pm0.18\times10^7$	(98.3)
2.93	24	70	$1.59\pm0.19\times10^4$	$3.09\pm0.46\times10^7$	(100)
2.93	32	265	$6.98\pm0.74\times10^4$	$2.43\pm0.17\times10^7$	(99.7)

[1]Determined from well water bacterial abundance and porosity data.
[2]Determined from porosity data and abundances of adherent bacteria in core samples taken adjacent to screens of sampled wells.

Table 18. Average distribution of aquifer solid material and adherent bacteria among four size fractions and portions lost after 2 minutes of settling during a preparative procedure used in enumeration of soils bacteria

Size fraction (μm)	Relative distribution[1]		Lost during 2 minutes settling[1]	
	Particulates (percent)	Adherent bacteria (percent)	Particulates (percent of size class)	Adherent bacteria (percent of total population)
<20	0.97±0.65	67.3±7.61	38.8±6.8	26.1±5.45
20-60	0.95±0.66	22.8±5.75	92.5±2.6	21.1±5.35
60-105	1.03±0.65	9.85±3.93	99.1±1.1	9.76±3.90
>105	97.1±1.89	0±0	100±0	0
Totals:	100	100	--	57.0±8.58

[1]Average and standard error computed for six core samples taken from contaminated and uncontaminated zones in the Cape Cod aquifer.

also shown in table 17. The free-living counts in the water samples were multiplied by the measured porosity of the corresponding cores to convert them to counts per unit volume of aquifer. A majority of the bacteria in all samples were attached to particulates. Portions of the total sample population associated with solid surfaces ranged from 98 percent at 0.21 km from the beds and 19 m below land surface to 100 percent at 2.93 km and 24 m. Population densities of adherent bacteria ranged from $1.84 \pm 0.22 \times 10^{7}$ cm^{-3} to $6.06 \pm 0.57 \times 10^{7}$ cm^{-3} of aquifer material, whereas population densities of free-living bacteria varied from $1.59 \pm 0.19 \times 10^{5}$ cm^{-3} to $6.42 \pm 0.66 \times 10^{5}$ cm^{-3}. Variations in abundances of adherent bacteria at 0.21 km correlated with changes in specific conductance, as did numbers of free-living bacteria in adjacent well samples (fig. 48). However, no significant differences were found between abundances of adherent bacteria for the two sites where cores were collected.

Average distributions of the adherent bacterial population among particle-size fractions in the six core samples are listed in table 18. A majority of the adherent bacteria (67 ± 7 percent) were associated with particles smaller than 20 µm diameter, which constituted only about 1 percent of the total particulate mass of the sample. In contrast the largest particulate size-fraction constituted a majority (97±2 percent) of the total particulate mass, but harbored an insignificant portion of the adherent bacterial population.

Portions of particulates and adherent bacteria that would settle out from well-mixed suspensions during 2 minutes of quiescent settling are also given in table 18. Most of the particulate mass in the three largest size fractions and greater than 38 percent of the particles less than 20 µm in diameter settled out of solution within 2 minutes. The total quantity of particulate matter settling out during this procedure corresponded to a loss of 57±9 percent of the adherent bacterial population from suspension. Much of the population loss was accounted for in the size fractions with diameters less than 60 µm.

Heterotrophic Uptake

Uptake rates of glucose in four ground-water samples taken between 0.64 and 1.8 km from the sand beds are illustrated in figure 49. Significant increases in uptake at both in-situ and 5 µM glucose concentrations were observed as distance from the source of contamination decreased. Uptake rates in glucose-amended (5µM) samples were 10.6 and 35.9 $nM \cdot day^{-1}$, for contaminated ground water from wells located 1.80 and 0.64 km from the beds, respectively. Uptake rates were more comparable when corrected for differences in the sizes of the bacterial populations. Specific uptake rates in amended samples collected from wells at 1.80 and 0.64 km were 3.36×10^{-17} and 2.47×10^{-17} $Moles \cdot bacterium^{-1}$ day^{-1}, respectively.

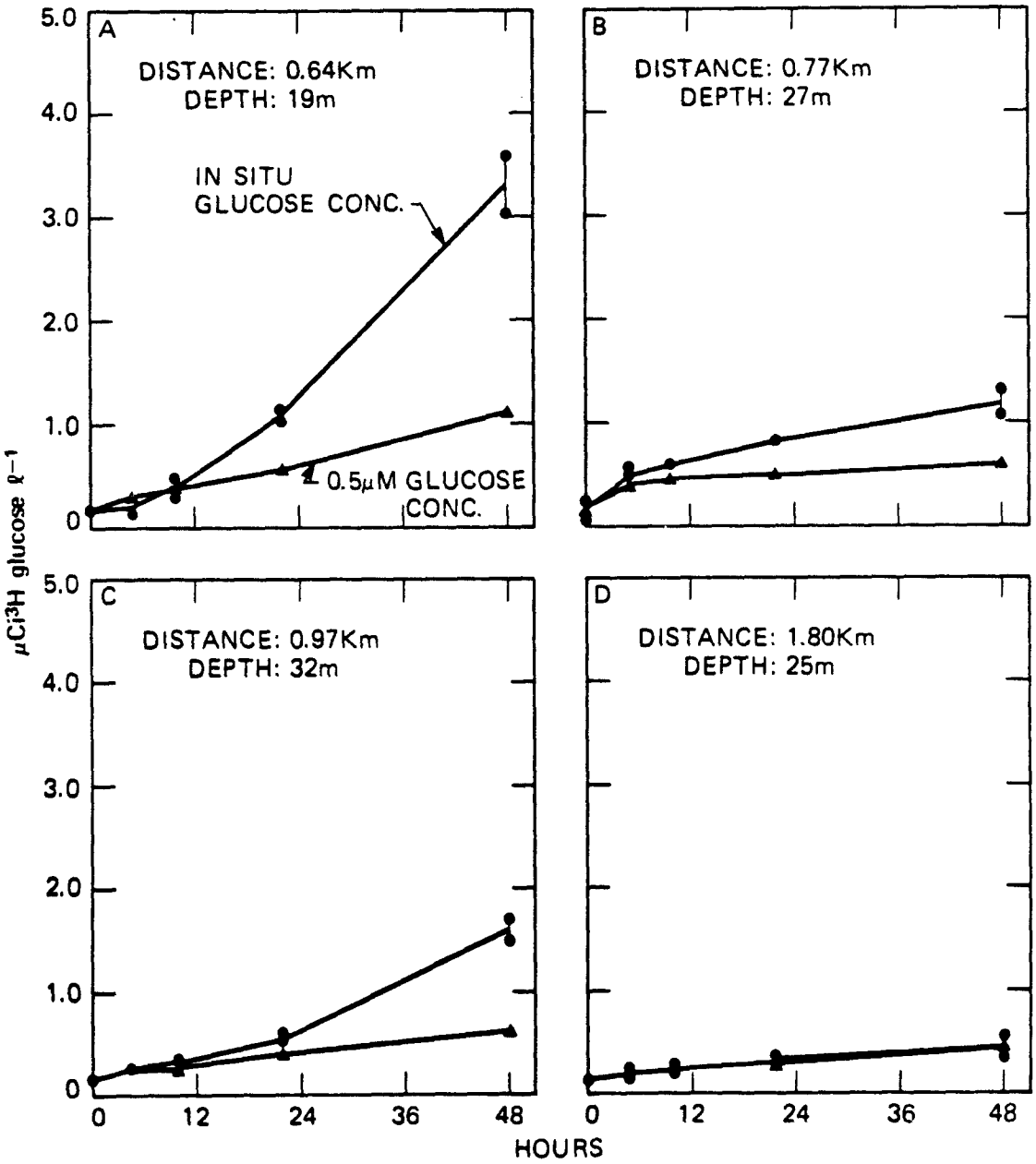

Figure 49. Uptake of ^{3}H-glucose versus time for four contaminated ground-water samples taken along the path of the plume at distances of 0.64 and 1.80 kilometers from the sewage-disposal area. Uptake of glucose in killed controls (not shown) was not significant.

RELATION OF BACTERIA TO PROXIMITY TO CONTAMINATION SOURCE

The strong correlation between abundance of free-living ground-water bacteria and proximity to the source of contamination (fig. 47) within 1 km of the infiltration beds suggests an influence from contaminant organics. This is also corroborated by data on glucose uptake rates, which were generally higher for contaminated ground-water samples taken closer to the source of contamination. However, much of the observed differences in glucose uptake rates among glucose-amended samples may be explained by differences in the sizes of the bacterial populations. Since ^{3}H-glucose autoradiography was not performed, uptake rates could not be expressed in terms of numbers of bacteria involved in active uptake of glucose. Nevertheless, these data suggest that bacterial populations in the plume closer to the infiltration beds may utilize readily-degraded organic compounds at higher rates than populations further downgradient.

The lack of correlation between numbers of free-living bacteria in contaminated ground water farther than 1.0 km from the beds and proximity to the infiltration beds (fig. 47) may reflect the more refractory nature of dissolved organic material (DOM) in this portion of the plume. Much of the DOM assayed in contaminated ground water sampled from the more distant observation wells consisted largely of highly-refractory detergents (Thurman and others, 1984). Since bacterial abundances in these samples are still significantly higher than those observed in uncontaminated samples from the same region, the plume may still have a measureable effect upon microbial biomass at distances of 2 to 3 km from sewage disposal site.

The variation in specific conductance and bacterial abundance in ground water sampled in the two depth profiles (fig. 48) illustrates the three-dimensional nature of the plume. The linear relationship between specific conductance and abundance of free-living bacteria appears to be an indirect one. Since specific conductance of the treated sewage (400 µmhos) is 4 to 5 times higher than the specific conductance of uncontaminated ground water (50 to 80 µmhos), observed variations in specific conductance within vertical transects through the plume reflect the degree of dilution with uncontaminated ground water. Therefore, specific conductance indirectly reflects dilution of other contaminants which do influence bacterial abundance. The free-living bacterial counts of $0.04 \pm 0.003 \times 10^{6}$ mL^{-1} observed in the uncontaminated ground-water sample (specific conductance equals 68 µmhos), collected from a well 0.21 km from the beds and 6 m deep, were similar to those found in another uncontaminated sample (specific conductance equals 70 µmhos; free-living bacterial counts equal $0.04 \pm 0.005 \times 10^{6}$ mL^{-1}) from a well 2.93 km from the beds and 24 m deep. These data suggest that bacterial abundance in uncontaminated ground water of this region may be as low as 10^{4}-10^{5} mL^{-1}. Reported bacterial abundances for other uncontaminated ground water range from 1.1×10^{3} to 2.0×10^{6} mL^{-1} (Olson and others, 1981; Ladd and others, 1982). These data were reported for samples taken at depths of 1200 m and 1.5 m, respectively, and thus may represent extreme values of population densities.

The microbial population in contaminated and uncontaminated areas of the saturated zone (table 18) appears to be dominated by adherent bacteria. Bacterial distributions between ground water

and particulate surfaces have not been reported for freshwater aquifers. However, large fractions of total bacterial populations associated with particulate surfaces in other particle-laden environments have been well documented (Harvey and Young, 1980a and 1980b; Harvey and others, 1983; Rublee and others, 1983) and seem reasonable for the terrestrial subsurface. Expressed in terms of particulate dry weight, the range of adherent bacteria reported here ($1.1\pm0.1\times10^{7}$ g^{-1} (counts per gram) to $3.4\pm0.3\times10^{7}$ g^{-1}) are somewhat higher than the range reported for six uncontaminated cores taken from the unsaturated and saturated subsurface at depths of 1.2 to 5.0 m in Oklahoma ($3.4\pm2.6\times10^{6}$ g^{-1} to $9.8\pm1.3\times10^{6}$ cells g^{-1}) (Wilson and others, 1983). These differences may, in part, reflect differences in enumeration techniques, since a settling procedure was used to separate out the larger sized particles in the latter study. Numbers of bacteria reported in the two studies for the saturated zone are three to four orders of magnitude lower than those reported for estuarine surface sediments (Rublee, 1982; Dale, 1974), likely reflecting substantial differences in available nutrients and environmental conditions.

It appears that most bacteria in the aquifer are associated with fine silt particles less than 20 µm in diameter (table 18). Furthermore, microscopic observations indicate a large number of bacterial clusters within the smaller particle-size fractions. This suggests a high degree of heterogeneity on a microscopic scale. The possibility exists that small colonies of bacteria associated with the silt-sized particle fraction may break down a significant quantity of the contaminant organics degraded in the aquifer, although more definitive study is clearly needed.

A substantial loss of particles smaller than 105 µm in diameter from solution during 2 minutes of settling suggests that a preparative settling procedure for enumeration of soils bacteria may not account for a substantial portion of the adherent bacterial population (table 18). Much of that loss occurs during the settling out of silt particles. It appears that wet sieving would likely yield more representative bacterial counts for subsurface samples. Since the procedure definitively separates out particulates greater than 105 µm in diameter, counts made of adherent bacteria in a variety of core material may be more comparable. The advantages would appear to outweigh the added investment of time and equipment required by the wet-sieving technique.

SUMMARY

The presence of organic contaminants in the ground water of Cape Cod significantly affects abundance and uptake potential of the free-living bacterial population. Numbers of free-living bacteria in well water samples varied with distance from the contaminant source and correlated with both specific conductance and glucose uptake rates. A majority of the bacteria in sampled portions of the aquifer appear to be associated with solid surfaces, as has been observed in other particle-laden systems. Although there is considerable heterogenity in abundance of adherent bacteria on a microscopic scale, surprisingly little

variation in abundance of adherent bacteria was observed among core samples. Clearly, more work is needed to better characterize the population of particle-bound bacteria in the aquifer and to delineate its role in the degradation of organic contaminants. Such studies would greatly benefit from improvements in sampling techniques for obtaining intact aquifer material from a variety of depths in the terrestrial subsurface.

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CHAPTER G

A major goal of research on toxic wastes in ground water is to determine the rates of microbial degradation and transformation of contaminants. This paper presents a preliminary study of one process, denitrification, which may play a significant role in the removal of nitrogen from ground water in the plume. Determination of the rates of denitrification and other nitrogen-transforming processes may be a first step toward simulation of nonconservative solute transport.

PRELIMINARY STUDY OF DENITRIFICATION IN A PLUME OF SEWAGE-CONTAMINATED GROUND WATER

by

Richard L. Smith and John H. Duff

ABSTRACT

High concentrations of nitrate (1 mM) were present in secondarily treated sewage entering sand infiltration beds at the Otis Air Base, Massachusetts, sewage-treatment plant, as opposed to undetectable concentrations of nitrate and oxygen in the underlying ground water. This suggested that a zone of denitrification had been established within the contaminated aquifer. Well water and core samples taken from the zone of contamination were assayed for denitrification potential using the acetylene blockage technique. Sewage effluent and slurried core material demonstrated endogenous denitrifying activity, whereas corresponding well water samples did not. Addition of nitrate (1 mmole/L) had no effect upon denitrification rates but glucose + nitrate amendments (1 mmole/L of each) stimulated activity in both water and core samples. In general, the highest rates of activity were evident in samples taken nearest the sand beds; samples taken from outside the contamination plume did not produce N_2O *under any conditions tested. These results establish that denitrification can occur in subsurface systems and thereby serve as a mechanism to remove nitrate from contaminated ground water.*

INTRODUCTION

The quality of ground water and the processes that affect the quality of ground water are topics of vast economic importance due to the widespread usage of subsurface aquifers for commercial and domestic purposes. Ground-water quality can be affected by physical-chemical interactions between soluble and particulate components of the aquifer material, by biological activities, and by anthropogenic influences. While each of these three components can have a direct bearing upon the others, in many cases the ultimate quality of ground water may be controlled by the activities of microorganisms within the ground-water system (McNabb and Dunlap, 1975).

Among the substances entering ground-water systems from anthropogenic origin, nitrate is very prominent. It is highly soluble and mobile and represents a health hazard at relatively low concentrations (Foster and others, 1982). Contamination of ground-water supplies by nitrate is an increasingly common phenomenon in both rural and urban areas as a result of natural and chemical fertilizer application and leaching of human and animal wastes (Foster and others, 1982; Gormly and Spalding, 1979; Katz and others, 1980; Porter, 1980; Robertson, 1979; Saffigna and Keeney, 1977; and Spalding and others, 1978). In agricultural regions, nitrate contamination can occur in widespread areas due to combined fertilization and irrigation practices (Saffigna and Keeney, 1977; Spalding and others, 1978).

Once it enters a ground-water supply, nitrate will likely remain in the ground water unless it is removed or transformed by biological activity. Reduction of nitrate to N_2 (denitrification) or to NH_4^+ (dissimilatory nitrate reduction) are energy-generating processes carried out by microorganisms and,as such, are the processes most likely to affect nitrate concentrations in ground water. These are anaerobic processes and hence would predominate only in O_2-free aquifers, but they also can occur in anaerobic microniches in habitats that are considered aerobic (Brock, 1979). Indirect evidence, such as NO_3^- and N_2 concentrations (Vogel and others, 1981; Robertson, 1979), $^{15}N/^{14}N$ ratios (Gormly and Spalding, 1979) and isolation of denitrifying bacteria (Willis and others, 1975; Whitelaw and Rees, 1980), suggest that

denitrification does occur in ground water. However, very little is actually known about denitrification rates in these habitats, even though such measurements are necessary to understand the dynamics and the controls of the process. The potential for nitrate reduction to ammonia has been virtually ignored in studies of aquifers and indeed is poorly understood in other habitats. However, this process can account for 7 to 30 percent of the nitrate reduced in lake sediments, 20 to 70 percent in marine sediments, and 60 to 70 percent in sewage sludge (J. M. Tiedje, Michigan State Univ., oral communication, 1982) and therefore must be considered as a potential nitrate sink in ground water as well. Thus, although these two processes represent potential detoxification mechanisms for ground-water supplies, the occurrence and extent of these activities in either contaminated or pristine aquifers is currently unknown.

The U.S. Geological Survey's ground-water contamination study site at Cape Cod, Massachusetts[1], represents a suitable location to study inorganic nitrogen transformations in ground-water systems. High concentrations of nitrate (1 mM) are present in the treated sewage percolating into the ground water, yet immediately downgradient from the infiltration beds the ground water contains no detectable nitrate or oxygen and high levels (0.6 to 0.7 mM) of ammonia (Ceazan and others, 1984). These trends suggest that a nitrate sink exists within the aquifer; denitrification and dissimilatory nitrate reduction are the most likely explanations for the nitrate disappearance. This paper describes the preliminary results of a study to test the hypothesis that a zone of denitrification and (or) dissimilatory nitrate reduction is established in an aquifer in response to increased quantities of nitrate and organic carbon and thereby serves as a mechanism to remove nitrate from ground water.

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[1]A description of the site, including its geologic and hydrologic setting, is given in Chapter A of this volume.

OVERVIEW OF DENITRIFICATION

Denitrification is an anaerobic process in which nitrate substitutes for the oxygen utilized in aerobic respiration. Nitrate acts as the terminal electron acceptor and is reduced in a stepwise fashion to dinitrogen gas:

$$NO_3^- \rightarrow NO_2^- \rightarrow (NO) \rightarrow N_2O \rightarrow N_2$$

Although the pathway involves gaseous intermediates (NO and N_2O), neither accumulate to a significant extent. Accurate estimates of denitrification activity by measuring the amount of N_2 produced are difficult to obtain due to the significant potential for interference by atmospheric N_2 (Knowles, 1982). However, the enzyme that catalyzes the terminal reduction of N_2O to N_2 , nitrous oxide reductase, is inhibited by acetylene. This phenomenon is utilized in a relatively simple and sensitive assay for denitrification known as the acetylene block technique (Balderston and others, 1976; Yoshinari and others, 1977; Knowles, 1982). Provided a sufficient acetylene concentration is present, the inhibition by acetylene is complete and results in the stoichiometric production of N_2O. Nitrous oxide is assayed by a sensitive gas chromatographic method that employs an electron capture detector (Kaspar and Tiedje, 1980). This technique has been utilized to estimate rates of denitrification in soils, sediments (both freshwater and marine), lake waters, digested sewage sludge and benthic stream communities (Kaspar and others, 1981; Knowles, 1982; Triska and Oremland, 1981).

EXPERIMENTAL PROCEDURES

Sample Collection

The study area consists of a sand and gravel aquifer located in Cape Cod, Massachusetts, which has been contaminated with secondary sewage effluent from the sewage treatment facility at Otis Air Base (fig. 50). The nature of the aquifer, the types of monitoring wells, and the contamination plume have been described in the preceding chapters. Observation wells

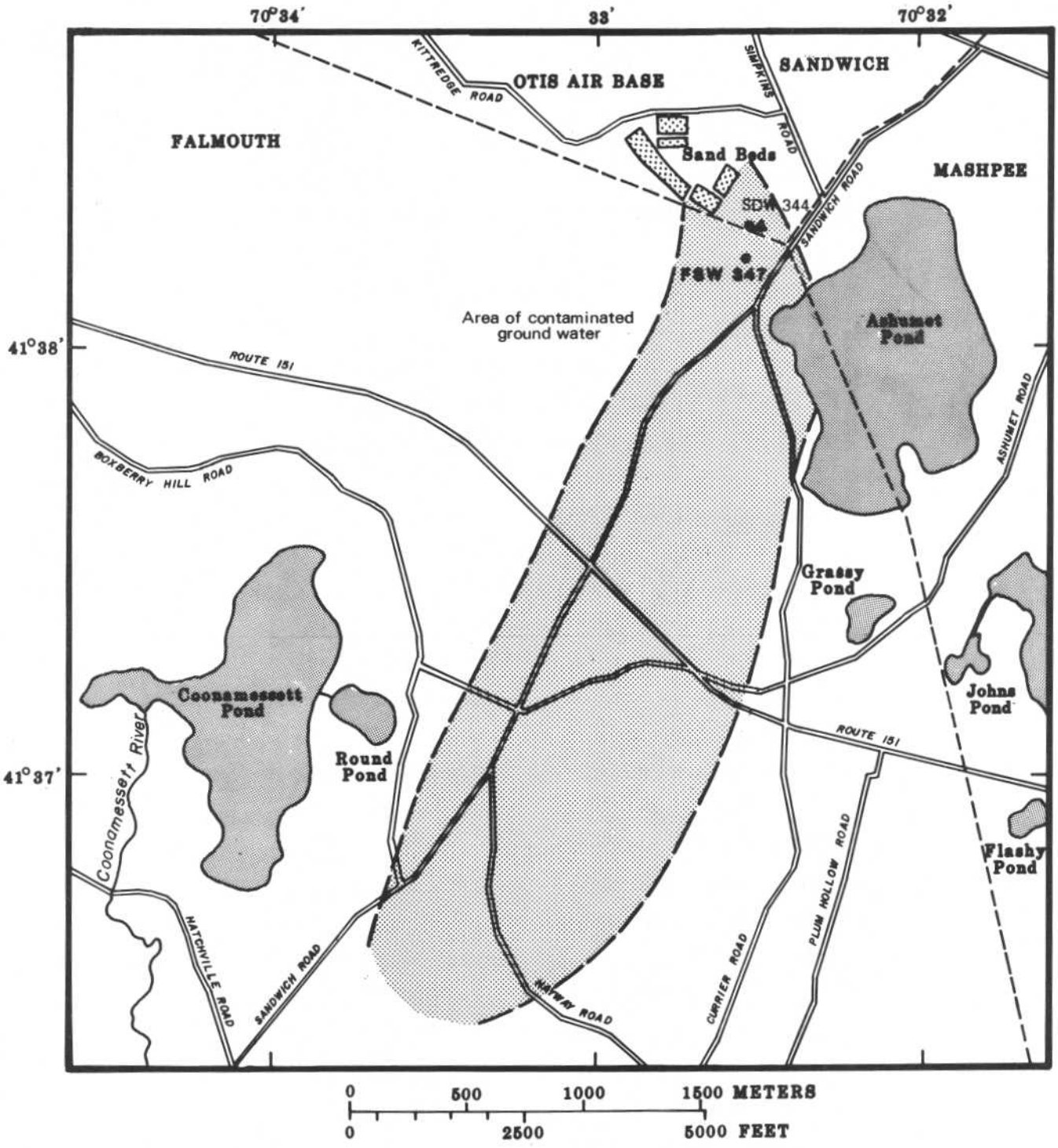

Figure 50. Ground-water study area in Cape Cod, Massachusetts. Dashed lines delineate the path of the contaminant plume (as determined by specific conductance) caused by infiltration of treated sewage into the aquifer. Triangle indicates site where core sample was taken. Circles indicate sampled observation wells.

were sampled with a Keck[2] submersible pump after suitable evacuation of the water contained within the well casing (Thurman and others, 1984). Specific conductance and dissolved oxygen were assayed as described by Ceazan and others (1984), dissolved methane as described by Oremland and Des Marais (1983), and ammonia and nitrate by a Hach water analysis kit. Water samples were collected in 1-liter acid-washed glass bottles that were completely filled and capped to avoid an air headspace. Secondarily treated sewage effluent was sampled at the Otis Air Base sewage-treatment plant from a flow channel leading onto an active infiltration sandbed. Aquifer sediments were sampled with an auger drilling rig immediately adjacent to well cluster SDW 344. Split-spoon cores (2 feet in length) were taken at selected depths (59-61, 78-80, and 98-100 feet below land surface) and the core material transferred to widemouth acid-washed glass jars. These jars were completely filled with sample material. All samples were stored on ice and transported to the U.S. Geological Survey laboratory in Menlo Park, California. Denitrification assays were initiated 2 to 3 days after sampling.

Denitrification Assay

Three 100 mL aliquots of each water sample were transferred to 150 mL serum bottles that were being flushed with O_2-free N_2. A fourth bottle received a 100 mL aliquot that had been filter sterilized with a 0.2 micron Gelman Acrodisc filter. The bottles were sealed with recessed butyl rubber stoppers and flushed with N_2 for an additional minute. A similar series of bottles was prepared for each core sample. Approximately 50 g (wet weight) of core material was transferred to pretared bottles. The solids were slurried with 50 mL of water sample taken from the well of corresponding depth. The bottles were flushed with O_2-free N_2 and reweighed. Control slurries were killed by adding $HgCl_2$ (1 percent weight per unit volume). All bottles were incubated at 12°C with shaking. After a 10-minute preincubation, two non-control bottles from each group of four was amended with 1.0 mL of 100 mM $NaNO_3$. One of the two bottles also received 1.0 mL of 100 mM glucose. Acetylene (10 mL) was added to the

[2]Use of trade names in this report is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey

headspace of all the bottles and the bottles were shaken for 2 minutes prior to the removal of zero-time samples. Successive headspace gas samples (0.3 mL) were removed by glass syringe and analyzed for N_2O by ^{63}Ni-electron capture gas chromatography (Culbertson and others, 1981).

RESULTS AND DISCUSSION

The specific conductance of ground-water samples taken from clusters of wells located 600 feet and 750 feet from the infiltration beds was 250 μmhos, or greater, with two exceptions (table 19). The exceptions were the shallowest well at each location. A comparison of these values with the specific conductance of the sewage effluent (390 μmhos) and an uncontaminated ground-water sample (51 μmhos) clearly indicates that these two well series represent depth profiles into the zone of contaminated ground water. The shallow wells are located near the upper periphery of the contamination zone, which in general is being displaced downward with increasing distance from the sandbeds by recharge from precipitation (LeBlanc, 1982).

The concentration of nitrate in the sewage effluent was high relative to the nitrate contained in uncontaminated ground water (table 19). However, the large nitrate inputs were not reflected in the nitrate profiles from either well series SDW 344 or FSW 347. Assuming that 1 mM represents an average long-term estimate of the sewage nitrate concentration, a mechanism for nitrate removal must exist in the ground-water system. Conversely, the ammonia concentration in several of the wells sampled was higher than that of the sewage effluent (table 19), indicating the potential for ammonia production within the aquifer as well.

At the location of well series SDW 344 and FSW 347 the contamination plume is anaerobic. Dissolved oxygen was detected only in water from well SDW 344-38, but at a concentration that was markedly lower than the dissolved oxygen content of uncontaminated ground water (table 19). The presence of dissolved methane corroborates the anaerobic nature of these ground-water samples since methane is produced by obligately anaerobic bacteria. Thus, the occurrence of a nitrate depletion, ammonia production,

Table 19. Chemistry of water samples taken from the contaminated ground-water study site.

Sample location or well number [a]	Distance from sewage beds (ft)	Specific conductance (μmhos)	Dissolved oxygen[c] (mg/L)	Dissolved methane (nM)	Nitrate (mM)	Ammonia (mM)
Sewage effluent	0	390[b]	5.0[b]	---	1.0	0.1-0.4
SDW 344-38	600	158	2.5	6	---	----
-61	600	380	<0.2	540	0	0.7
-80	600	250	0.5	460	0	0.6
-100	600	350	<0.2	470	0	0.6
FSW 347-20	750	37	<0.2	0	0.06	0.02
-46	750	325	<0.2	12	0	0.6
-67	750	345	<0.2	110	0	0.2
-101	750	260	<0.2	22	0.003	0.2
FSW 242-51[d]	6200	51	9.9	---	0.01	0

[a]The number after the dash is the depth (feet) of the well screen below the land surface.

[b]Data taken from LeBlanc (1982).

[c]The lower limit of detection of the oxygen electrode utilized was 0.2 mg/L.

[d]Water from this well is assumed to represent uncontaminated ground water.

and anaerobic conditions suggest that these two locations within the contamination zone were potential sites for active denitrification and dissimilatory nitrate reduction.

Denitrifying activity in water samples from well SDW 344-100 was assayed with the acetylene blockage technique (fig. 51). No N_2O production was detected during 60-hour incubations with an unamended subsample (endogenous), a subsample amended with 1 mmole/L NO_3^-, or a filtered control. However, a subsample amended with 1 mmole/L glucose + 1 mmole/L nitrate did display significant N_2O production after a 35-hour time lag. This denitrification potential implies the presence of denitrifying bacteria in this well water sample. This population was either inactive (with respect to denitrification) or present in very low numbers in the unamended subsample, or both. The in situ nitrate concentration does not appear to be the limiting factor since the nitrate amendment did not stimulate denitrification. However, the response to the addition of glucose plus nitrate identified the availability of electron donors as a factor controlling denitrification. Indeed, the 35-hour time lag and the exponential increase in the rate of N_2O production in this subsample strongly imply active growth of a denitrifying population during the course of this assay.

A similar set of denitrification assays were run with water samples from the other wells in the FSW 347 and SDW 344 series. N_2O was not produced with any of the unamended subsamples, with the exception of well SDW 344-61. In that sample 0.1 nmoles N_2O/mL H_2O was produced after 60 hours. The addition of 1.0 mmole/L NO_3^- had no effect on any sample. Figure 52 presents the results of the glucose + nitrate amendment. In general, samples from the two deeper wells at SDW 344 and the middle wells at FSW 347 (FSW 347-46, FSW 347-67) demonstrated the greatest stimulation. N_2O production in water from well FSW 347-20, which is not within the zone of contamination (table 19), was not stimulated by the addition of glucose and nitrate. This result, plus the minimal stimulation in samples from wells FSW 347-101 and SDW 344-61, indicates that the response of the samples that were stimulated was not due to the introduction of contaminants by the sampling and handling procedures.

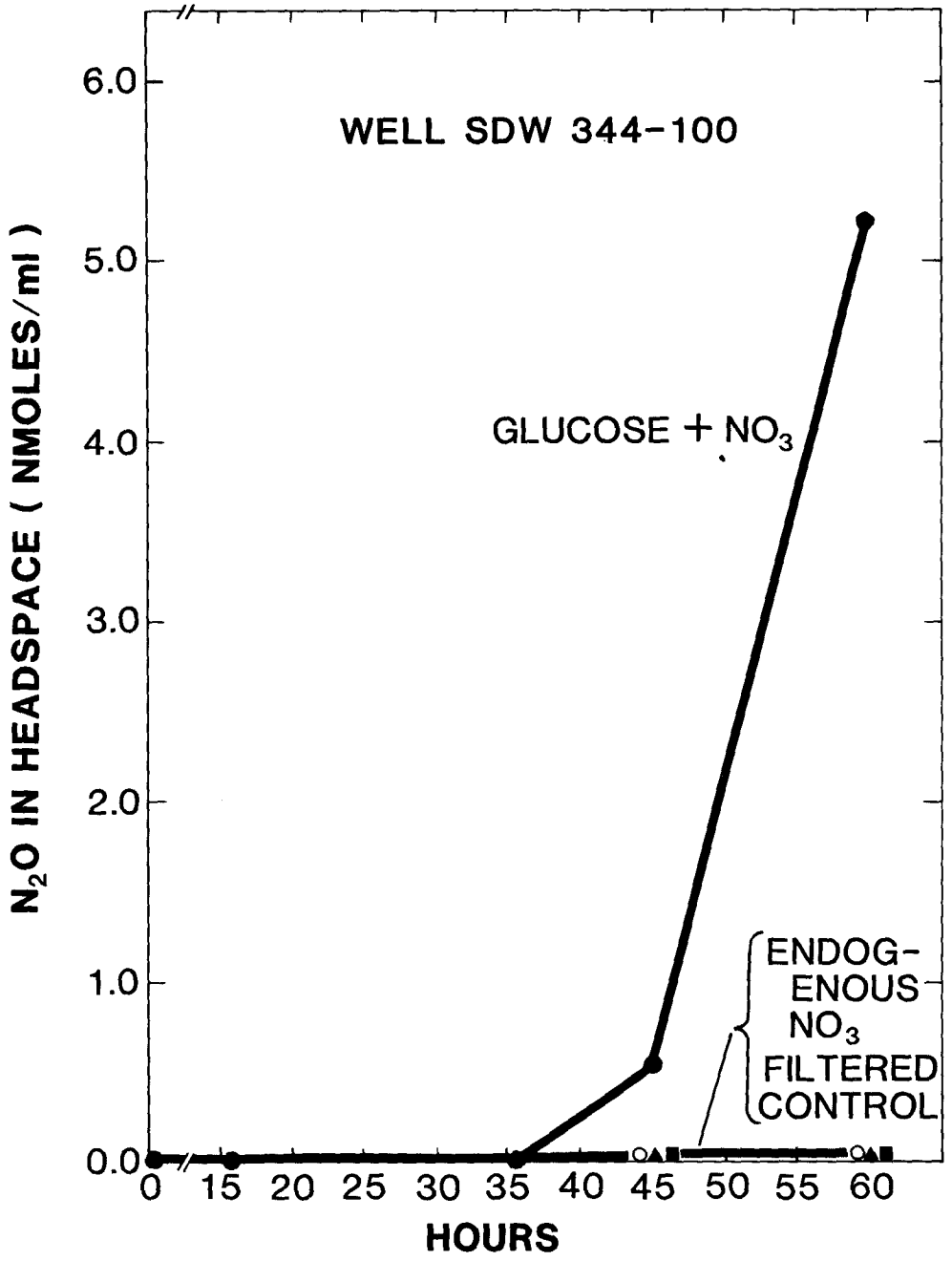

Figure 51. N_2O production in water from well SDW 344-100. Sample incubated at ambient nitrate concentration (o), amended with 1 mmole/L NO_3^- (▲), amended with 1 mmole/L NO_3^- + 1 mmole/L glucose (●), or filtered control (■).

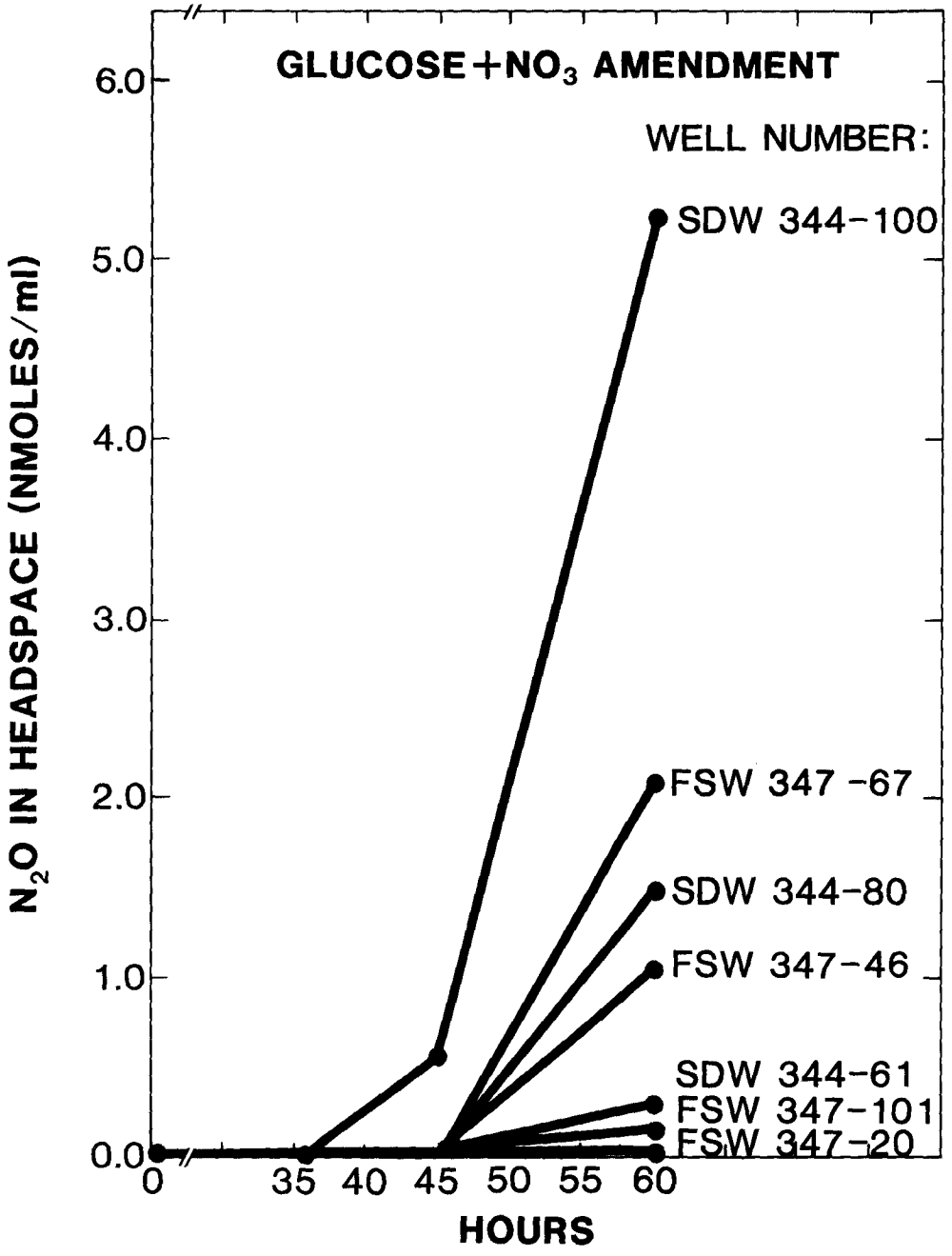

Figure 52. N_2O production in well-water samples when amended with 1 mmole/L NO_3^- + 1 mmole/L glucose.

A major hypothesis being tested at the Cape Cod contamination site is that a significant fraction of microbial biomass and microbially-mediated activity is associated with particulate surfaces rather than in a free-living state in the interstitial ground water (see Harvey and others, 1984). Hence, denitrifying activity was also assayed in core material taken opposite each well screen at SDW 344. Slurries prepared from core material taken from the 78-80 ft depth interval produced N_2O endogenously (fig. 53). The lack of N_2O production in the killed control ($HgCl_2$) indicated that the activity was biologically mediated. As with the well water samples, the addition of nitrate had no effect, whereas glucose + nitrate greatly enhanced the level of activity. In general, these trends were evident for each core (fig. 54 and 55). Nitrate stimulated N_2O production five-fold for the core material from 59 to 61 ft, which was the only exception (data not shown). Particularly significant was the fact that endogenous N_2O production was evident for each core sample, but not for the well water sample taken from the equivalent depth. These results suggest that core samples, and not well water samples, are required to adequately assess rates of denitrification in ground-water aquifers.

For comparative purposes, the sewage effluent entering the infiltration sand beds was also assayed for denitrification potential (fig. 56). Unlike the well water samples, the sewage effluent displayed a significant rate of endogenous activity, with no apparent time lag. Amendment with glucose + nitrate greatly stimulated the rate of N_2O production, also with little time lag. Thus, the sewage effluent must contain a sizeable population of microorganisms capable of denitrification, which is characteristic of waste treatment systems (Kaspar and others, 1981; Knowles, 1982). It should be noted however, that the sewage effluent is oxygenated (table 19), whereas the assay system is anaerobic. The actual rate of denitrification in the sewage effluent before it percolates into the sand bed is probably very low.

A summary of the denitrification rates for both water and core samples during the 60-hour incubation is presented in figures 57 and 58. Since the rate of N_2O production was not linear with time in most cases, the calculated rates are labeled as denitrification potentials. They do not

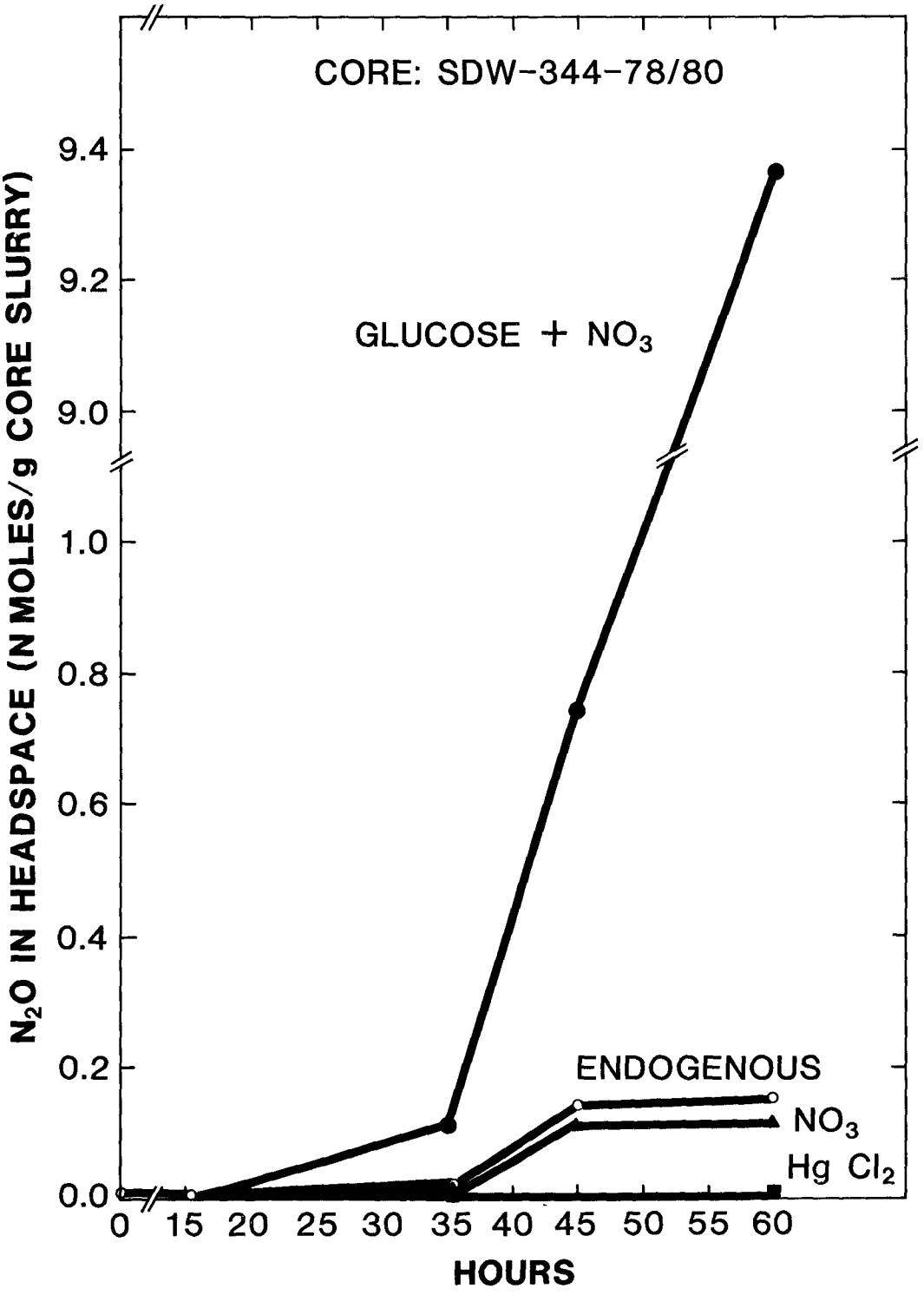

Figure 53. N_2O production in slurried core material taken from the 78 to 80 ft depth interval immediately adjacent to well series SDW 344. Sample incubated at ambient nitrate concentration (o), amended with 1 mmole/L NO_3^- (▲), amended with 1 mmole/L NO_3^- + 1 mmole/L glucose (●), or amended with $HgCl_2$ (■).

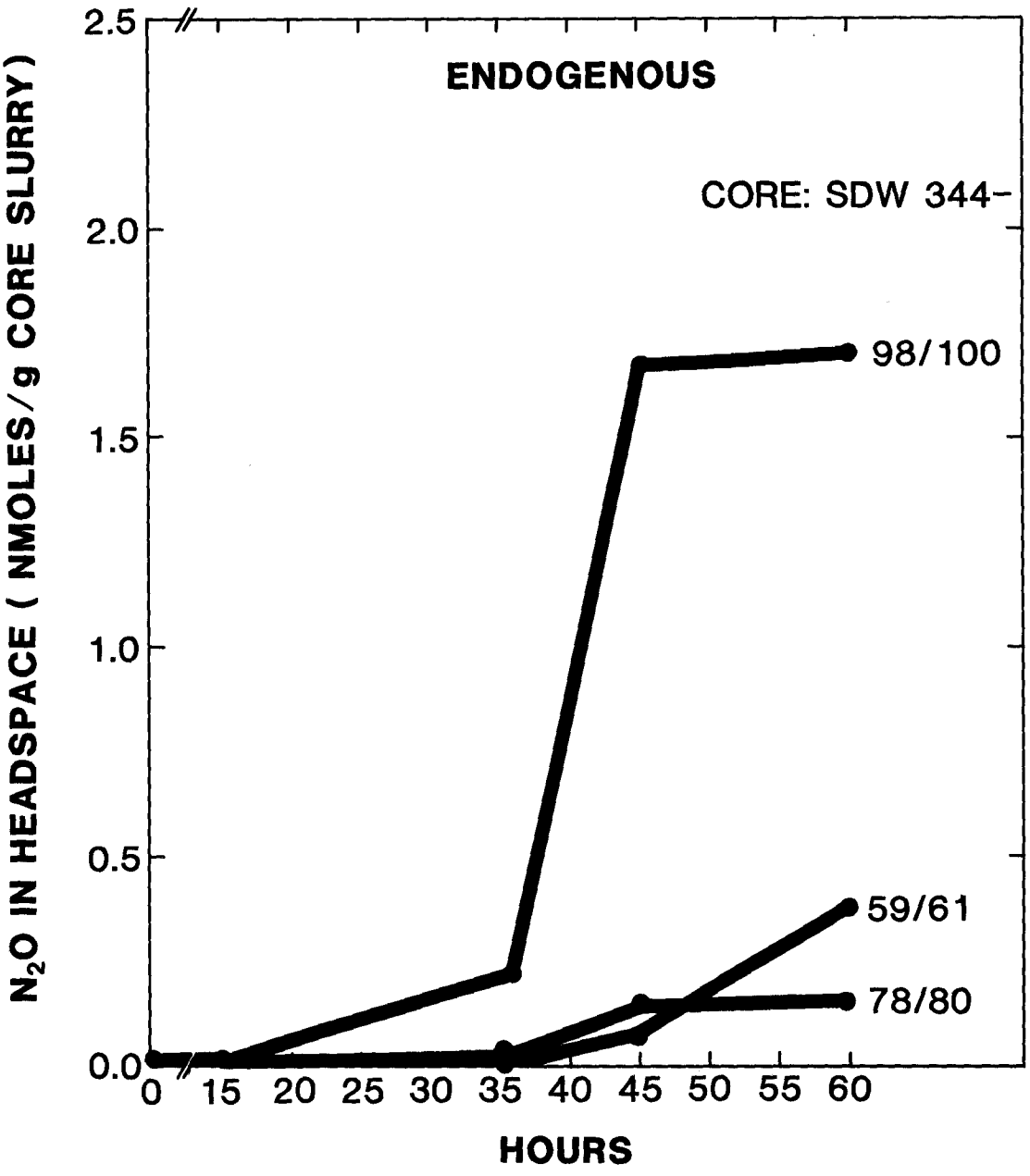

Figure 54. N_2O production in slurried core material from core SDW 344 when incubated at ambient nitrate concentration. Depth (ft) of the core from land surface as indicated.

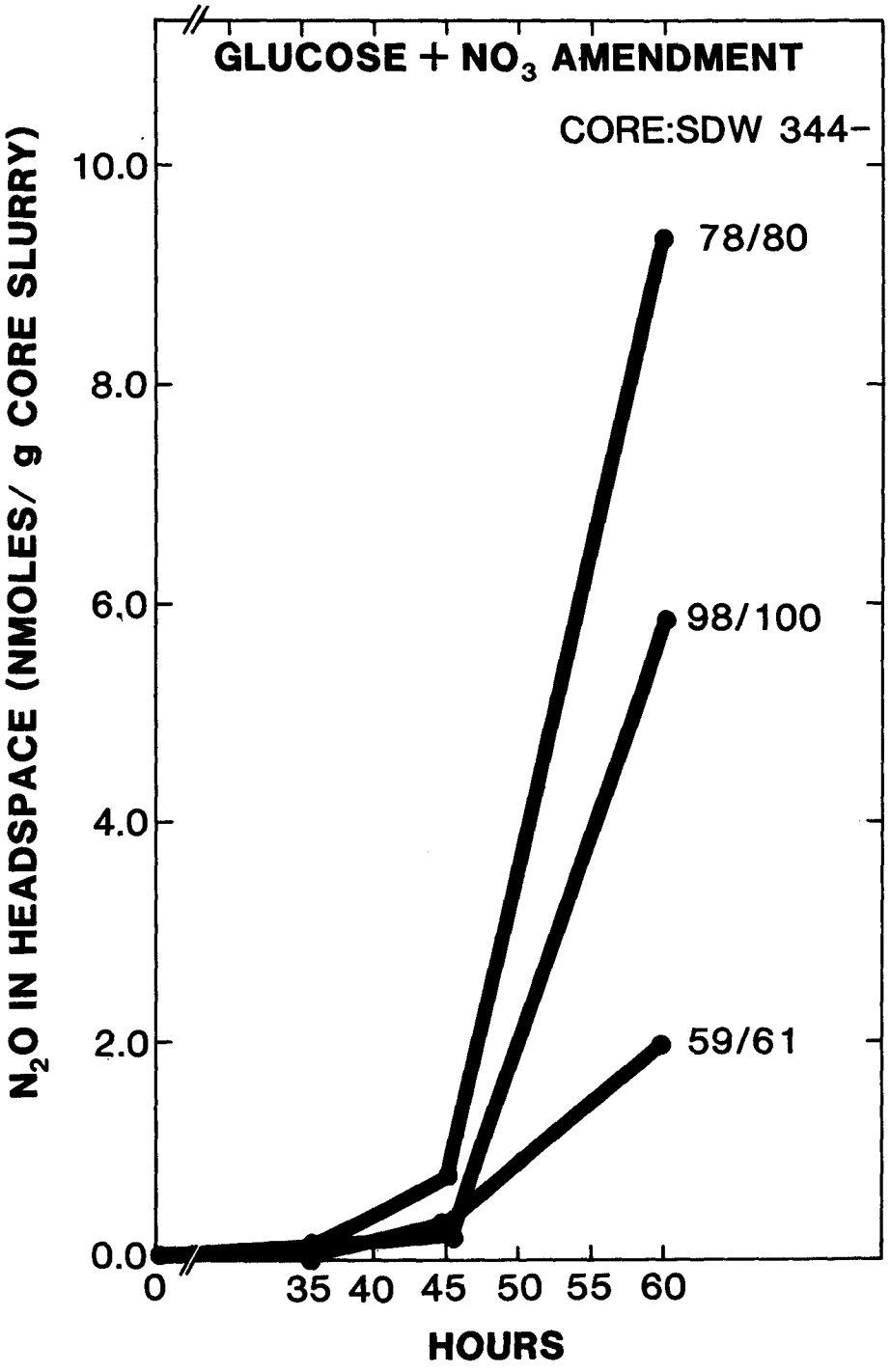

Figure 55. N_2O production in slurried core material from core SDW 344 when amended with 1 mmole/L NO_3^- + 1 mmole/L glucose. Depth (ft) of the core from land surface as indicated.

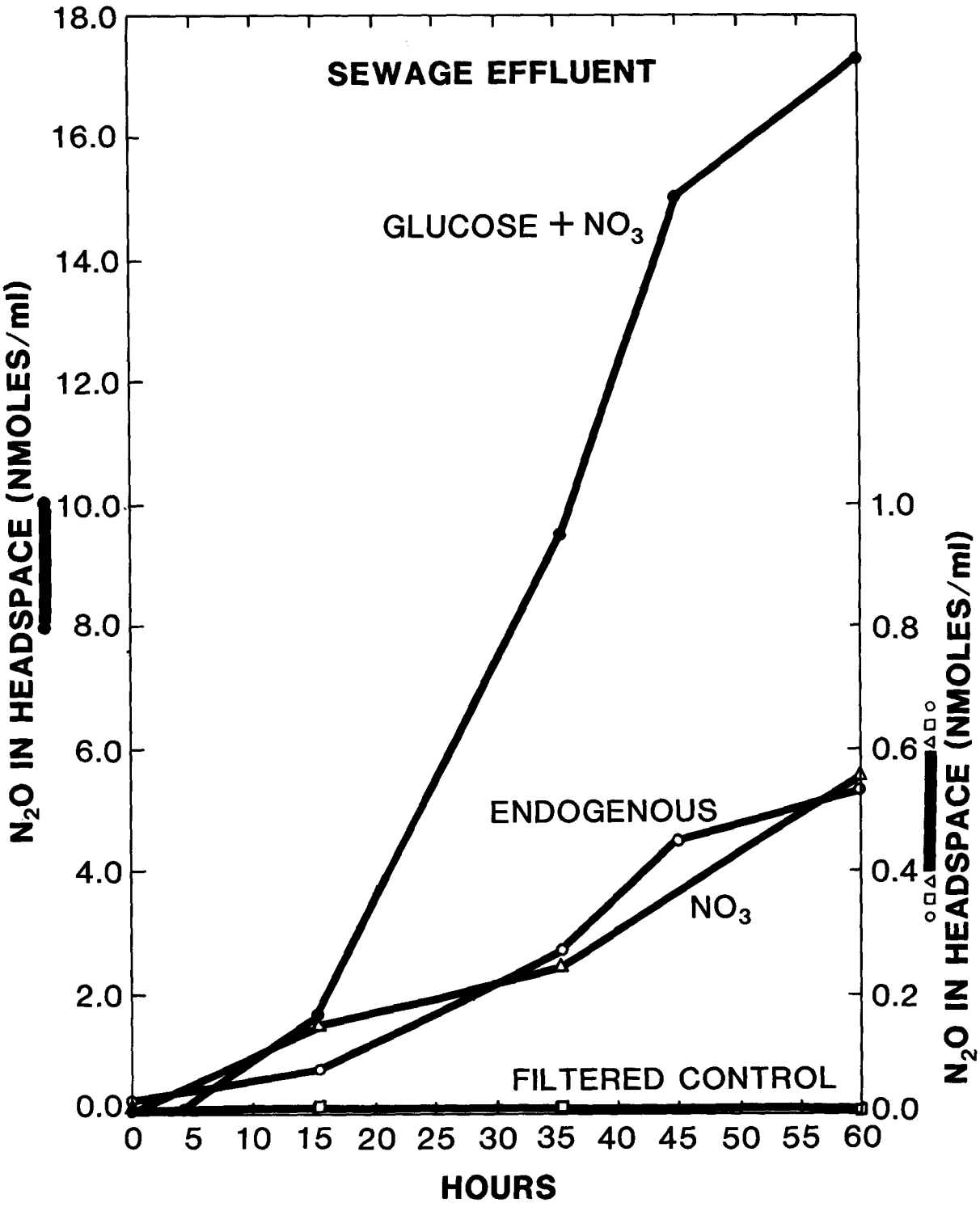

Figure 56. N_2O production by the sewage effluent entering an infiltration sand bed on December 8, 1983. Sample incubated at ambient nitrate concentration (o), amended with 1 mmole/L NO_3^- (Δ), amended with 1 mmole/L NO_3^- + 1 mmole/L glucose (●), or filtered control (□).

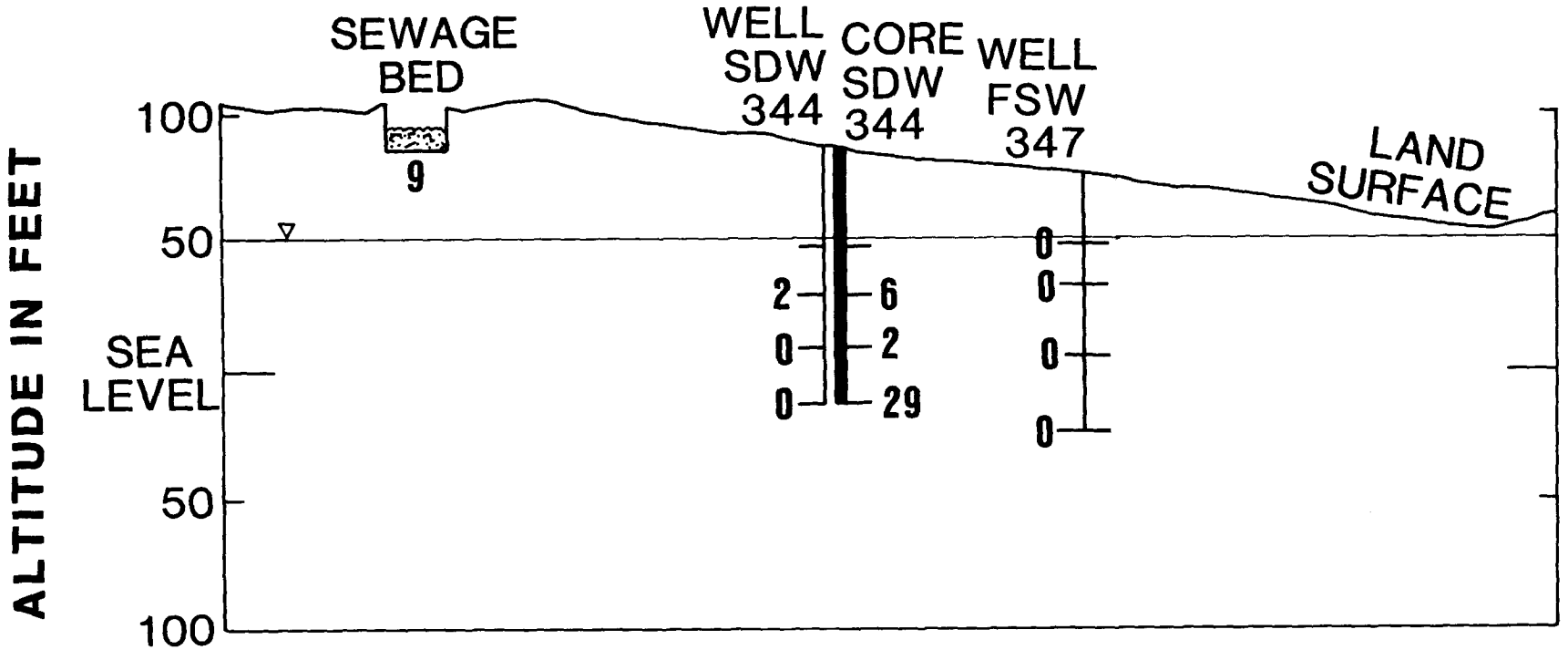

Figure 57. Vertical distribution of denitrification potential for samples incubated at ambient nitrate concentration. Rates were calculated from the 60-hour time point of time course assays. Rates are given in pmoles N_2O produced/ml H_2O/hr for water samples, and pmoles N_2O produced/g core slurry/hr for core samples. Horizontal axis not drawn to scale.

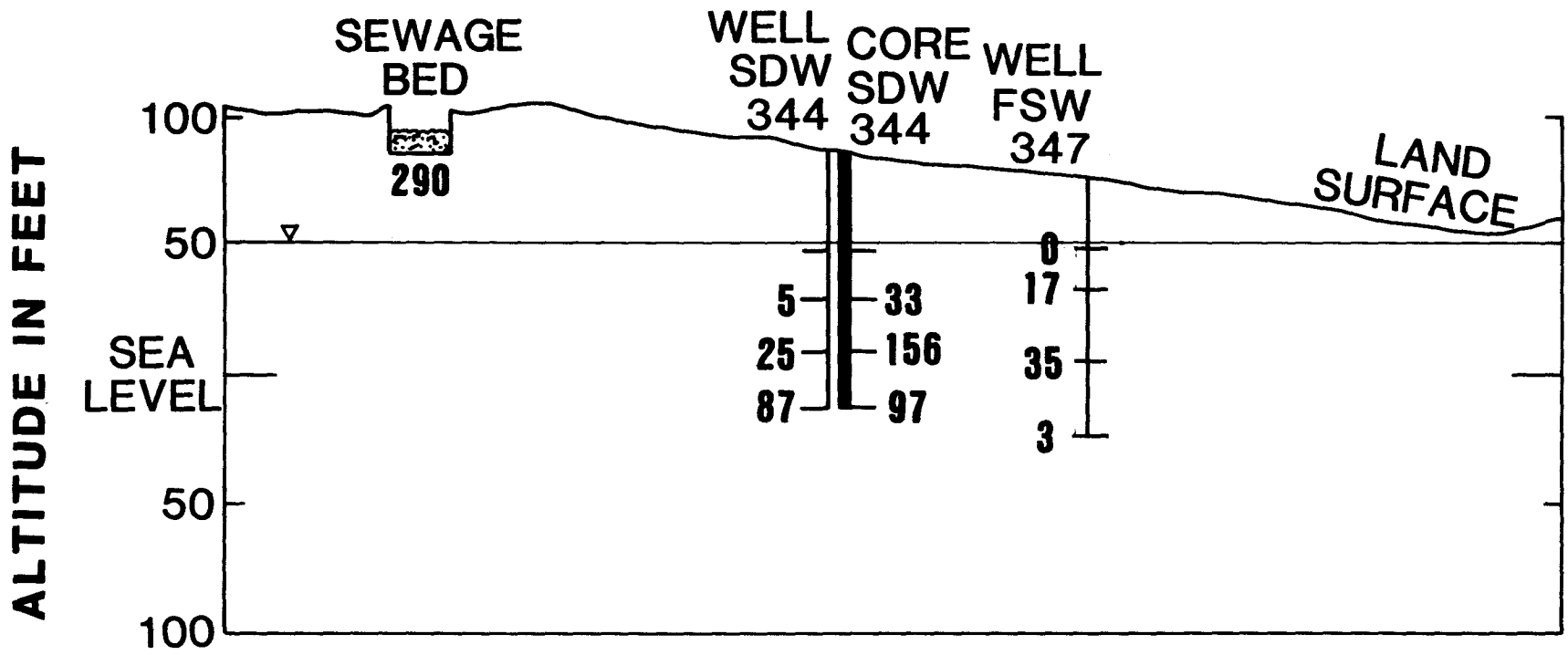

Figure 58. Vertical distribution of denitrification potential for samples amended with 1 mmole/L NO_3^- + 1 mmole/L glucose. Rates were calculated from the 60-hour time point of time course assays. Rates are given in pmoles N_2O produced/ml H_2O/hr for water samples or pmoles N_2O produced/g core slurry/hr for core samples. Horizontal axis not drawn to scale.

necessarily represent the actual rate in the ground-water system. However, these results demonstrate that denitrification can occur in ground-water systems and thereby serve as a mechanism for nitrate removal from ground water. The activity appears to be associated with solid surfaces within the aquifer. With one exception, no denitrification was evident in unamended well water samples (fig. 57). This has important ramifications for the sampling techniques employed in future studies involving microbial processes in ground water.

In general, denitrification in this environment seems to be carbon (electron donor) limited rather than nitrate limited. Additions of glucose stimulated denitrification in most water samples, all of the core samples, and even the sewage effluent (fig. 58), despite the relatively high levels of dissolved organic carbon present in the effluent (Thurman and others, 1984). On the other hand, denitrification was essentially non-existent in samples from wells FSW 347-20 and FSW 347-101 (fig. 56 and 57). Thus, the denitrifying activity demonstrated by most of the samples was not due to an indigenous population of denitrifying organisms present throughout the aquifer. Rather, a zone of denitrification has been established in response to the sewage contamination. This zone is not established solely by an elevated nitrate concentration. For example, well FSW 347-20 contained 0.06 mM NO_3^- and no dissolved oxygen (table 19). The results suggest that there is a requirement for sufficient electron donor availability as well. Indeed, once the denitrification zone becomes established, the nitrate concentration within that zone is actually lower than the nitrate concentration in the uncontaminated ground water (table 19).

This preliminary study raises several points which remain to be addressed. First, the potential for dissimilatory nitrate reduction to ammonia remains to be examined. This activity may be as significant as denitrification. Since the ammonia concentration in the well water is high, tracer techniques will have to be developed to assay this activity. Procedures for sampling core material also need to be developed to retain interstitial water and preclude potential sample contamination. In addition, denitrification assay techniques need to be refined to eliminate the time lag and obtain linear rates of N_2O production. Finally, it appears that the

maximum rates of denitrification are occurring much closer to the sand beds than originally anticipated. Given the potential denitrification rates in the sewage effluent, it is quite possible that most of the nitrate present in the effluent is actually reduced immediately beneath the sand beds. If such is the case, the system would be very efficient in its ability to remove the nitrate present in the sewage effluent.

SUMMARY AND CONCLUSIONS

1) High concentrations of nitrate present in sewage effluent and low concentrations of nitrate and oxygen in the sewage-contaminated ground water suggested that a zone of denitrification had been established within a contaminated aquifer at Otis Air Base, Cape Cod, Massachusetts.

2) Well water and core samples taken from the zone of contamination were assayed for denitrification potential using the acetylene blockage technique. The core material demonstrated endogenous denitrifying activity while the corresponding well water sample did not. This suggested that the denitrifying microorganisms were associated with particulate surfaces and would not be adequately quantified with well water samples.

3) Denitrification rates were stimulated by addition of glucose, but not by addition of nitrate, in both water and core samples, indicating that the activity was carbon-limited rather than nitrate-limited within this environment.

4) In general, the highest rates of denitrification occurred in samples taken from nearest the infiltration sand beds, while samples taken from outside the contamination plume did not produce N_2O.

5) These results establish that denitrification can occur in subsurface systems and thereby serve as a mechanism to remove nitrate from contaminated ground water.

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